AUG 11 1924

Subscription Price:
United States and Canada, $6.00 Per Year.
Great Britain, 25 Shillings; Single Copies, 3 Shillings.
Foreign, $6.00 Per Year; Single Copies, 75 Cents.

VOLUME VI AUGUST, 1924 NUMBER 4

Medical Library

# THE JOURNAL OF INDUSTRIAL HYGIENE

## AND ABSTRACT OF THE LITERATURE

OFFICIAL ORGAN OF
THE AMERICAN ASSOCIATION OF INDUSTRIAL PHYSICIANS AND SURGEONS

EDITOR, UNITED STATES
DAVID L. EDSALL, M.D., S.D.

HONORARY CONSULTING EDITOR
THOMAS M. LEGGE, M.D., D.P.H.

EDITOR, GREAT BRITAIN
E. L. COLLIS, M.D., M.R.C.S.

### Lead Studies V

### A. The Distribution of Lead in the Organism after Absorption by the Gastro-Intestinal Tract

### B. The Distribution of Lead in the Organism after Absorption by the Lungs and Subcutaneous Tissue

### C. The Distribution of Lead in the Human Organism

### Lead Studies X

### The Deposition of Lead Salts, with a Note on the Solubilities of Di-Lead Phosphate in Water at 25°C. and of Di-Lead and Tri-Lead Phosphates in Lactic Acid at 25°C.

PUBLISHED MONTHLY BY HARVARD MEDICAL SCHOOL
EDITORIAL AND BUSINESS OFFICES : : 55 VAN DYKE STREET, BOSTON 17, MASSACHUSETTS
GREAT BRITAIN : : INDUSTRIAL WELFARE SOCIETY, 51 PALACE STREET, WESTMINSTER, S. W. 1

Entered as second-class matter, at the Post Office at Boston, Mass., under Act of March 3, 1879. Published Monthly.
Copyright, 1924, by Harvard Medical School.

# *IMPORTANT NEW BOOKS*

## Insanity and Law

**This book states in simple language psychiatric facts regarded as established, using a nomenclature that has been adopted as a standard by almost all the hospitals for mental diseases in this country.**

**By H. Douglas Singer, M.D., M.R.C.P., Professor of Psychiatry, University of Illinois, and William O. Krohn, M.D., Ph.D., Formerly Head of Department of Psychiatry at Western Reserve University and at University of Illinois, etc.**

**Imitation Leather. $6.00**

## Industrial Health

**An Indispensable New Reference Work on Industrial Hygiene, Public Health, Safety Appliances, Maintaining Health in Industry; Cause and Preventive Measures; Standards. Prepared by Collaboration of 33 Authors. Edited by George M. Kober, M.D., LL.D., Washington, D. C., and Emery R. Hayhurst, A.M., M.D., Ph.D., Columbus, Ohio. With Illustrations, Tables, Appendix.**

**Cloth, $15.00**

## Public Health Administration

**Relation of Health Officer to Physician and Public; Organization, Function, Health Departments; Finance, Management; Births, Deaths, Heredity, Vital Statistics, Maternal Mortality, Infant Mortality; Hygiene, Child Hygiene; Water, Sewage Wastes, Milk, Foods, Education, Publicity, Laws, Social Welfare, State and Federal Departments; Preventable Diseases; Appendices; Nurse; Score Cards; Plans; Emergencies; Technique; Bibliography, etc.**

**By George Sparr Luckett, A.B., M.D., Director of Public Health, State of New Mexico, and Harold Farnsworth Gray, B.S., M.S., Gr.P.H., Chief, Division of Sanitary Engineering and Sanitation, New Mexico Bureau of Public Health.**

**Cloth, $3.00**

## Toxicology

**A concise description of the effects of poisons upon the organism.**

**Contents: Principles of Toxicology; Inorganic Poisons; Poisonous Gases; Metallic Poisons; Alkaloidal Poisons; Miscellaneous Organic Poisons.**

**By Frank P. Underhill, Ph.D., Professor of Pharmacology and Toxicology, Yale University Medical School.**

**12 mo. Cloth, $2.25**

## P. Blakiston's Son & Co., *Publishers*

1012 Walnut Street, Philadelphia

# THE JOURNAL OF INDUSTRIAL HYGIENE

PUBLISHED MONTHLY

VOLUME VI AUGUST, 1924 NUMBER 4


## LEAD STUDIES V

## A. THE DISTRIBUTION OF LEAD IN THE ORGANISM AFTER ABSORPTION BY THE GASTRO-INTESTINAL TRACT*


A. S. MINOT, PH.D.

*From the Laboratories of Physiology of the Harvard Medical School*


### *Introduction*

TYPICAL lead poisoning is a chronic intoxication which is characterized by marked latency of development and frequent recurrences. These features suggest a storage of lead in the body and make knowledge of the amount and localization of the lead retained in the organism of fundamental importance in understanding the disease. This has been recognized before; and since the introduction of quantitative chemical methods, many analyses of the tissues and excreta of poisoned men and animals have been made. Most of the available data are very fragmentary and incidental, while in the few extensive chemical studies unsatisfactory methods of analysis have produced conflicting and often inaccurate results. It appeared important, therefore, to reinvestigate the whole field, using a uniform method of known accuracy in order to determine (1) the importance of lead storage in the development of chronic poisoning, and (2) the localization of these stored deposits. A study of these problems is the purpose of the investigations reported in this paper.

### *Review of Literature*

One of the earliest chemical studies of lead distribution was made by Gusserow (1) in 1861. A pioneer in the use of electrolytic methods, this author deposited lead as a metal by electrolysis of a solution of ashed tissues. Confirmatory qualitative tests were made by converting this deposit to lead sulphide and by reducing the sulphide again to metallic lead. If sufficient quantities were found, the lead was then converted to the sulphate and weighed. Although crude, according to modern criteria, this method served to give an approximate knowledge of the relative concentrations of lead in various tissues. Rabbits poisoned by daily administration of 4 gm. of lead sulphate by


*Received for publication May 12, 1924.

mouth for twenty to twenty-five days were studied. Amounts of lead which could be weighed were found in the skeleton—6 to 8 mg. per 100 gm. of bone—while large traces were reported in muscles and kidneys, and small traces or none at all in the central nervous system. Selective localization of the lead in the skeleton was quite apparent, and this led Gusserow to suggest the possibility of the formation of double salts of lead and calcium in the bones. He also hinted that a migration of lead in and out of the bony deposit was possible and probably was conditioned by changes in the organism as a whole. From Gusserow's time to the present this idea apparently has been ignored, but it is of great interest in the light of the findings to be presented in this paper.

Contemporary clinical investigations were evidently much influenced by Gusserow's report that the muscles of poisoned animals contain a comparatively high concentration of lead. Without further experimental work several papers (2) appeared explaining such typical symptoms of lead poisoning as paralysis, colic, encephalopathy, and abortion as due to muscle lesions.

In 1871 Heubel (3) reported an extensive experimental study of lead poisoning. Using the gravimetric sulphate method, he studied the concentrations of lead present in the tissues of poisoned dogs. The relative concentrations reported are probably correct, but the limitations of this method of analysis have been discussed recently by Fairhall (4). The highest concentration of lead was found in the bones, with the kidney, liver, and central nervous system next in order. The lead content of muscle was found to be very low in contrast to the figures reported by Gusserow. Heubel maintained that, in general, the symptoms noted resulted from the selective action of lead on certain tissues. This depends on two factors, (*a*) the chemical affinity of the tissues for lead, and (*b*) the relative susceptibility to poisons. Thus the bones which have a high affinity for lead, but a low susceptibility, store the poison without becoming damaged. Heubel believed, moreover, that the well-known susceptibility of the central nervous system to poisons and the significant amounts of lead which he found present in brain and cord indicate that nerve changes as well as muscle lesions are involved in the syndrome of chronic plumbism.

Ellenberger and Hofmeister (5), using the same method of analysis, reported a similar localization of lead in the tissues of sheep poisoned by ingestion of lead.

A study reported in 1889 by Prévost and Binet (6) is in many respects the most complete in the literature. Acute and chronic lead poisoning were produced in large numbers of rats and guinea-pigs and in a few larger animals. The lead was separated as chromate and determined gravimetrically. The small amounts of lead found in the tissues made the application of a gravimetric method impractical in many instances, and in most cases only qualitative findings were reported. In acute poisoning following gastro-intestinal absorption the authors found the greatest amounts of lead in the liver; the kidneys and bones were next in order of concentration. More typical chronic poisoning, lasting from three months to a year, caused a marked localization in the skeleton of a large part of the absorbed lead. These authors believed that lead was deposited in the bones as a phosphate which, they predicted, might remain stored for long pe-

riods. Had larger animals been used and a titrimetric method substituted for the determination of the chromate precipitate, their work would have left little to be added to our knowledge of lead distribution.

Although other phases of chronic lead poisoning received considerable attention in the intervening period, the next important chemical studies were made by Meillère (7), who collected his results in a monograph in 1903. The electrolytic separation of lead as peroxide followed by a gravimetric determination was employed in most of his experimental work. Certain sources of error in electrolytic determination of lead in biological material have been discussed recently in a publication from this laboratory (8). In Meillère's hands, however, the method seems to have been carefully controlled and probably gave accurate results. He has contributed to the literature the most extensive study of human autopsy material in cases of lead poisoning. These data, together with an experimental study of the progressive selective localization of lead in bones and keratinous tissue in more chronic types of plumbism, make this work of particular interest in connection with the present study.

More recently Kisskalt and Friedmann (9) have made chemical studies of the tissues of rabbits poisoned with lead. The method of analysis was somewhat cumbersome, and the many manipulations carried out with the small amounts of lead involved must have introduced considerable error. These authors found large amounts of lead in the brains of their experimental animals—a finding which several investigators have reported but which we have been unable to duplicate.

The "lead stream" theory as the explanation of the fundamental mechanism of lead poisoning was developed by Straub (10) and Erlenmeyer (11). Both investigators injected lead carbonate or sulphate subcutaneously into cats. These compounds were gradually absorbed, and in the course of a few weeks the animals developed chronic poisoning. After death the lead content of the excreta, of the unabsorbed remainder of the deposit, and of the bodies of the animals was determined by the gravimetric sulphate method. Most of the absorbed lead was found in the excreta. The tissues, aside from those in the immediate region of the subcutaneous deposit, contained only very small amounts of lead. No attempt was made to determine the quantity in individual organs. The inaccuracies of this analytical method for the determination of small amounts of lead, and the study of only one type of plumbism furnished very inadequate experimental data. Nevertheless, the deductions made by Straub and Erlenmeyer from these data suggest an explanation for the action of lead in the organism.

Their experimental animals had been severely and often fatally poisoned during the process of transportation of lead from the site of injection to the organs where it was subsequently excreted, although at the time of death only very small amounts of lead were found stored in the organism. These authors believed that toxic effects are due, not to deposits of lead at the site of injury, but rather to the "lead stream," that is, to the small concentration of soluble lead which is transported in the circulating blood. They pointed out that the "stream" is always small—too small to produce any effect in a single dose—but that as absorption and transportation go on, innumerable subminimal attacks bring about pathological changes in certain

sensitive tissues; and that both time and the concentration of this "lead stream" are important factors in the production of toxic symptoms.

A recapitulation of the various contributions mentioned in this brief survey of the literature gives two general impressions: (*a*) that lead tends to become localized and stored in the bones, and (*b*) that the continued presence of traces of soluble lead in the blood stream, rather than a storage of lead at the site of injury, is probably responsible for the damage done. To mold this fragmentary evidence into a real explanation of the action of lead when absorbed into the organism, however, requires further study of the entire problem by means of accurate and uniform methods.

*Description of Method*

The titrimetric method described by Fairhall (4) can be readily applied to the determination of lead in most tissues. Some adaptation of the regular procedure proved necessary in the present investigation for separation of minute amounts of lead from the large bulk of calcium salts present in bone; hence a description of the exact method of determination of lead in the skeleton will be included here. The cleaned skeleton or specimen of bone is heated to dull red heat in an electric oven until the organic matter is destroyed and the bones can be powdered with mortar and pestle. Sufficient concentrated hydrochloric acid is added to this inorganic residue to form a thin paste. After the first active chemical reaction with calcium carbonate, approximately an equal volume of water is added, and the mixture is allowed to stand for twenty-four to forty-eight hours. Practically all the calcium phosphate is thus dissolved in a minimum amount of acid. Any undissolved residue is re-ignited with the filter paper, dissolved in acid, and added to the main solution. Obviously neutralization as carried out in more dilute solutions is impractical because the incidental precipitation of calcium phosphate would render further manipulation difficult. Certain factors, however, so co-operate to reduce the actual acidity that such neutralization is unnecessary. (*a*) During the process of solution the following reactions occur:

1. $Ca_3(PO_4)_2+4HCl \rightarrow CaH_4(PO_4)_2+2CaCl_2$
2. $CaH_4(PO_4)_2+2HCl \rightarrow CaCl_2+2H_3PO_4$

Thus, unless an unnecessary excess of hydrochloric acid has been added, practically all the free acid present in the resulting solution is phosphoric. This is a weak acid and has so little tendency to replace the lead in lead sulphide that a quantitative precipitation can be carried out in a 5 to 15 per cent. solution of phosphoric acid. (*b*) As sodium hydroxide is added to this acid solution of the skeleton in which a large proportion of the calcium is still present as $CaH_4(PO_4)_2$, this acid salt is neutralized as follows:

$$CaH_4(PO_4)_2 \rightarrow Ca_2HPO_4 \rightarrow Ca_3(PO_4)_2$$

To produce this change in the large bulk of calcium salt present requires a considerable amount of sodium hydroxide, but the change produced in the actual acidity of the solution, after the free hydrochloric and phosphoric acids are neutralized, is slight. In other words, most of the apparent acidity is due to the buffer effect of normal and acid calcium phosphates rather than to free acid. Hence, complete neutralization of the solution is not only impractical but unnecessary.

The solution of the skeleton is diluted with an approximately equal volume of

water to reduce the acid concentration and is then saturated with hydrogen sulphide gas. If the lead sulphide precipitate does not appear at once, 25 per cent. sodium hydroxide is added drop by drop while the solution is shaken vigorously. Before any appreciable amount of calcium phosphate is thrown out of solution, the lead sulphide precipitates completely. This first precipitate of lead sulphide is, however, contaminated with small amounts of lead and calcium phosphates. These are removed by solution in hydrochloric acid and the lead is subsequently reprecipitated as sulphide. From this point the analysis may be made as Fairhall describes.

Satisfactory recoveries of quantities of lead ranging from 0.1 to 100 mg., both from pure solutions and when added to normal tissues, have been made repeatedly by this method. To save space only one series of recoveries is reported here. In these tests an attempt was made to involve all the difficulties encountered in actual analyses of biological material in order to ascertain the accuracy of the method under the conditions of the actual experiments. Known amounts of lead acetate were fed to rats, and after several days the lead was recovered by separate analyses of the excreta and bodies of the animals. Table 1 indicates that the method is sufficiently accurate and adaptable to give reliable data even with the widely different concentrations of lead present in the various tissues of the body. Of course, when the amount of lead in the body is determined by means of from ten to fifteen separate analyses of individual tissues, the total error unavoidably increases. This total error, however, rarely exceeds 5 per cent., and the distribution pictures obtained are true.

TABLE 1.—RECOVERIES FROM TISSUES AND EXCRETA OF LEAD FED TO RATS

| NUMBER | AMOUNT OF LEAD FED | LEAD RECOVERED | | | | |
|---|---|---|---|---|---|---|
| | | IN TISSUES | IN EXCRETA | TOTAL | ERROR | |
| | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *%* |
| 1 | 127.92 | 4.63 | 122.06 | 126.69 | 1.23 | 0.93 |
| 2 | 91.20 | 3.22 | 87.18 | 90.40 | 0.80 | 0.87 |
| 3 | 167.00 | 6.13 | 151.20 | 157.30 | 9.70 | 5.80[1] |
| 4 | 143.40 | 33.70 | 109.50 | 143.20 | 0.20 | 0.14 |
| 5 | 118.15 | 5.87 | 111.70 | 117.57 | 0.58 | 0.50 |
| 6 | 105.70 | 2.53 | 103.48 | 106.01 | 0.31 | 0.30 |
| | | | | Average Error | 0.62 | 0.55 |

[1] Known loss during manipulation; not included in average.

### *Description of Experiments*

Whatever may be the effect of lead entering by other routes, everyone agrees that lead compounds taken into the gastro-intestinal tract are gradually absorbed and, if continually ingested, sooner or later cause plumbism. Carlson and Woelfel (12) have shown that even those compounds of lead which are considered most insoluble dissolve to some extent in the gastric juice. Meillère believes that the entire gastro-intestinal tract absorbs soluble lead, although in the alkaline medium of the small intestine and in the hydrogen sulphide often present in the colon a large

percentage of lead is precipitated. This portion, together with what escapes solution in the stomach, represents a large part of the total ingested lead and probably never truly enters the organism, but is excreted directly in the feces. Enough is absorbed, however, even from very small continuous doses, to cause poisoning. The distribution in the body of this absorbed lead is the subject of the investigation here reported.

Acute lead poisoning following gastro-intestinal absorption is rare except in cases of attempted suicide or after large doses of lead compounds taken to produce abortion. To differentiate rigidly between acute and chronic saturnism is difficult (11) (13), for if a patient survives the first large dose, excretion of the absorbed lead may often be so slow that the acute intoxication develops into chronic poisoning. Hence, no arbitrary division of cases will be made in this paper.

After some preliminary experimentation it was found that chronic poisoning could be produced in cats without the production of acute gastric symptoms by the administration on alternate days of lead acetate solution in doses of 50 mg. of *lead* per kilo of body weight. The animals in this group of experiments died or were killed after varying periods of administration of lead, and the lead distribution in the body was then studied. In every instance the entire body was analyzed, the lead content of the various tissues being determined separately and calculated as percentage of total lead. These figures give a more instructive picture of the geographical distribution of the total lead in the body than do the concentration figures presented by earlier investigators. The following procedures were adopted in routine analysis. The contents of the gastro-intestinal tract were always removed, and these tissues were carefully washed before analysis. Whether the lead in the contents was unabsorbed or excreted, it certainly could not be considered part of the lead actually within the organism at the time of death. A portion of fresh muscle was analyzed to determine the concentration in lead; and the total amount present in muscle was found by analyzing all meat boiled from the bones. Such a figure, of course, represents not pure muscle alone, but also cartilage, fat, connective tissue, etc., which could not be separated satisfactorily. The error introduced by this, however, is slight, for the amount of lead present is in most cases a small and unimportant part of the total. In calculating the totals, lead found in the skin and hair has been consistently omitted, even though in many instances the amount had been determined. This was done because most, if not all, of the lead in the skin of experimental animals seemed due to contamination. Thorough washing of the fur removed a large part of the lead, and since, after analyzing the skins of many animals, we obtained no consistent results to support Meillère's (14) theory of a migration of lead to keratinous tissues in chronic cases, it was decided to regard the small and variable amounts of lead found as external contamination rather than as absorbed lead.

Separate protocols best show the varied individual reactions to lead feeding.

PROTOCOL 1.—Rabbit A. Female, weight 3.6 kg.

*Feb. 27, 1922.* 1 gm. lead acetate by mouth.
*March 4, 1922.* 1 gm. lead acetate by mouth.
Animal very dull and sick. Loss of appetite complete.

Many stippled red cells in blood.
*March 9, 1922.* Animal died.

Duration of experiment, 9 days.
Total lead received, 1.09 gm.

PROTOCOL 2.—Cat C. Female, weight 3.0 kg.

The blood vessels of the left kidney in this animal ligated several weeks previous to this experiment. The cat completely recovered from this operation and in good condition at beginning of administration of lead.

*Nov. 15, 1923.* First administration of lead.

*Nov. 26, 1923.* No marked symptoms of lead poisoning except lead line. Distemper.

*Nov. 27, 1923.* Severe distemper. Animal killed. Weight 2.2 kg.

Duration of experiment, 12 days.
Total lead given, 1.35 gm.

PROTOCOL 3.—Cat D. Female, weight 4.3 kg.

*Nov. 3, 1922.* First administration of lead.

*Nov. 6, 1922.* Appetite poor.

*Nov. 15, 1922.* A few atypical stippled red cells in blood.

Diarrhea and frequent vomiting.

*Nov. 20, 1922.* Mild symptoms of distemper.

*Nov. 21, 1922.* Animal killed. Weight 3.4 kg.

Duration of experiment, 18 days.
Total lead given, 2.58 gm.

PROTOCOL 4.—Cat E. Female, weight 3.4 kg.

*Jan. 21, 1922.* First administration of lead.

Prompt loss of appetite, appearance of lead line, stomatitis, and muscular weakness.

*Feb. 20, 1922.* Animal died. Very emaciated, weight 1.8 kg.

Duration of experiment, 30 days.
Total lead given, 2.80 gm.

PROTOCOL 5.—Cat F. Female, weight 3.3 kg.

*Jan. 21, 1922.* First administration of lead.

Salivation, marked lead line, occasional vomiting, and extreme muscular weakness. A very few atypical stippled red cells in blood.

*March 5, 1922.* Animal died. Weight 2.4 kg.

Duration of experiment, 43 days.
Total lead given, 3.78 gm.

PROTOCOL 6.—Cat G. Male, weight 4.6 kg.

*Oct. 4, 1921.* First administration of lead.

*Oct. 24, 1921.* Decreased appetite, marked thirst.

*Nov. 4, 1921.* No appetite. Dulness. Lead line.

*Nov. 23-28, 1921.* Given 4 gm. potassium iodide in four days. Cat very sick. Marked salivation due to potassium iodide.

*Nov. 29, 1921.* Animal died. Weight 2.8 kg.

Duration of experiment, 55 days.
Total lead given, 3.74 gm.

PROTOCOL 7.—Cat H. Male, weight 4.0 kg.

*Feb. 10, 1922.* First administration of lead—75 mg. per kilo of body weight.

Frequent vomiting. Animal's mouth in bad condition, teeth discolored, gums bleed easily. Prompt development of a marked lead line. Marked continuing loss of appetite. Refusal of practically all food for ten days before death.

*April 18, 1922.* Animal died. Weight 2.4 kg.

Duration of experiment, 67 days.
Total lead given, 7.11 gm.

PROTOCOL 8.—Cat I. Female, weight 3.7 kg.

*Oct. 4, 1921.* First administration of lead.

*Nov. 1, 1921.* Loss of appetite. Little food taken.

*Nov. 8, 1921.* Very few atypical stippled red cells in blood.

*Nov. 25, 1921.* Definite lead line. No typical paralysis. Marked muscular weakness. Loss of appetite the most marked symptom throughout. Extreme emaciation.

*Jan. 7, 1922.* Animal died. Weight 1.6 kg.

Duration of experiment, 95 days.
Total lead given, 4.72 gm.

PROTOCOL 9.—Cat J. Male, weight 5.5 kg.

*Oct. 4, 1921.* First administration of lead. No abnormal symptoms during first month.

*Nov. 5, 1921.* Appetite poor and rather variable. Animal somewhat dull and sickly, and very constipated. A marked lead line and a few somewhat atypical stippled red cells in blood. Definite poisoning. Death directly due to lung infection rather than to plumbism.

*Jan. 11, 1922.* Animal died. Weight 4.5 kg.

Duration of experiment, 99 days.
Total lead given, 11.40 gm.

TABLE 2.—COLLECTED DISTRIBUTION FIGURES FOR TEN ANIMALS POISONED BY GASTRO-INTESTINAL ROUTE

| ANIMAL | WEIGHT | | LENGTH OF EXPERIMENT | TOTAL LEAD | | PERCENTAGE DISTRIBUTION OF ABSORBED LEAD IN TISSUES | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | START | END | | GIVEN | IN BODY | SKELETON | MUSCLE | LIVER | KIDNEY | STOMACH | SMALL INTESTINE | COLON | HEART | LUNGS | BRAIN AND CORD | SPLEEN | OMENTUM |
| | *kg.* | *kg.* | *days* | *gm.* | *mg.* | % | % | % | % | % | % | % | % | % | % | % | % |
| Rab. A | 3.6 | 3.4 | 9 | 1.09 | 5.85 | 47.3 | 0.0 | 29.7 | 6.0 | 5.1 | 4.0 | 5.5 | 1.4 | 1.4 | 0.0 | ... | ... |
| Cat C | 3.0 | 2.2 | 12 | 1.35 | 12.86 | 81.5 | 5.0 | 6.3 | 2.1 | 3.2[1] | | | ... | 1.9 | 0.0 | ... | ... |
| Cat D | 4.3 | 3.4 | 18 | 2.58 | 21.85 | 40.9 | 12.4 | 21.8 | 11.3 | 5.2 | 3.6 | 2.0 | 1.2 | 1.7 | 0.0 | 0.0 | 0.0 |
| Cat E | 3.4 | 1.8 | 30 | 2.80 | 37.08 | 84.4 | 10.3 | 0.6 | 0.5 | 1.4 | 1.3 | 1.5 | 0.6 | trace | 0.0 | ... | ... |
| Cat F | 3.3 | 2.4 | 43 | 3.78 | 11.24 | 69.9 | 6.4 | 6.6 | 1.4 | 3.9 | 2.6 | 2.2 | 3.8 | 0.0 | 3.2 | ... | ... |
| Cat G | 4.6 | 2.8 | 55 | 3.74 | 16.48 | 65.8 | 1.7 | 7.9 | 5.4 | 7.0 | 1.2 | 0.4 | 7.0 | 1.4 | 0.4 | 0.5 | 0.6 |
| Cat H | 4.0 | 2.4 | 67 | 7.11 | 23.90 | 39.0 | 20.5 | 14.7 | 13.6 | 5.0 | 0.8 | 3.0 | 0.8 | 1.8 | 0.6 | ... | ... |
| Cat I | 3.7 | 1.6 | 95 | 4.72 | 23.00 | 47.2 | 4.5 | 20.6 | 6.1 | 2.2 | 6.1 | 2.5 | 0.9 | 1.8 | 1.2 | 1.6 | ... |
| Cat J | 5.5 | 4.5 | 99 | 11.40 | 86.33 | 77.2 | 12.8 | 7.4 | 0.3 | 0.2 | 0.2 | 0.6 | 0.3 | 0.3 | 0.1 | ... | 0.6 |
| Cat K | 3.4 | 1.8 | 124 | 6.28 | 88.99 | 85.5 | 4.0 | 3.6 | 2.4 | 1.5 | 0.1 | 1.6 | 0.4 | 0.2 | 0.2 | ... | .. |

[1] Gastro-intestinal tract as a whole.

PROTOCOL 10.—Cat K. Female, weight 3.4 kg.

*Nov. 3, 1922.* First administration of lead.

Appetite very variable from the beginning, vomiting frequent. Teeth so carious that meat eaten with difficulty.

*Feb., 1922.* Gradual development of paralysis of the left forepaw, analogous to wrist drop in man.

Animal very weak.

*March 8, 1922.* Dissection of the brachial nerves on both sides under ether anesthesia. Stimulation with interrupted current from induction coil. Very weak response on left side, normal response on right side.

Animal bled to death under anesthesia. Weight 1.8 kg.

Duration of experiment, 124 days.
Total lead given, 6.28 gm.

In Table 2 are assembled figures showing the percentage distribution of lead in these ten animals. Figures 1 and 2 show the distribution in cats D and J.

A comparison of the figures in Table 2 is of more value than a summary since the wide variation in the distribution of lead in individual animals emphasizes that changes in localization of lead occur at different stages of poisoning. The amount of lead retained in the skeleton ranges from 39 to 85.5 per cent. of the total deposit in the body. In every case this is the largest percentage held by any single tissue, and in some cases this selective localization is so marked that the skeletal deposit represents a majority of all the lead stored. In general this localization increases with time and becomes very marked in the more chronic cases; but striking exceptions, such as cats H and I, indicate that time is not the only factor involved.

The total weight of lead stored in the body ranges from 5.8 to 88.9 mg. This is a surprisingly small figure to follow the ingestion of from 1.0 to 11.4 gm. of lead in the form of lead acetate and indicates that some extremely efficient mechanism prevents the absorption of lead by the general circulation. The well-known fact that the liver efficiently removes foreign material from the blood stream, as well as the experimental data in Table 2, suggests that

the protective mechanism is to be found in this organ. In shorter experiments, the liver contains a relatively high percentage of the total lead absorbed. This is derived from the portal circulation. The fact that the quantity of lead stored in the liver does not increase progressively as absorption continues, and the fact already noted, that the total retention of lead in the body remains small, are evidences of extraordinarily efficient excretion by the liver. Brady (15) furnishes further experimental proof of this in work which shows the bile to be an important path of excretion for lead. Here, then, we have a mechanism which protects the organism by a retention in the comparatively invulnerable region of the intestines, liver, and portal circulation of a large proportion of the lead absorbed through the gastro-intestinal tract. A small amount of lead, however, by escaping excretion in the bile, enters the general circulation and becomes distributed throughout the organism. Immediately the great avidity of bone for lead carried in the blood stream is apparent. Among the soft tissues the higher concentrations of lead are found in kidney, heart, and muscle. Apparently the bones tend to hold and store the lead removed from the blood stream. Hence a shifting of relative concentrations gradually occurs, and in the absence of complicating factors a progressively higher percentage of the total lead retained is held by the skeleton. This change is well illustrated by contrasting the much more general distribution of lead absorbed in the tissues of Cat D (see Fig. 1), which lived for eighteen days, with the marked selective localization in Cat J after 162 days of administration of lead (see Fig. 2).

The question at once arises whether, if absorption ceased, all the lead would become localized and stored in the bones as a temporarily harmless deposit. A series of experiments was, therefore, carried out to test this point. Lead was given by mouth to six cats, in the doses described in the preceding experiments, until the animals were severely poisoned. Just before death seemed

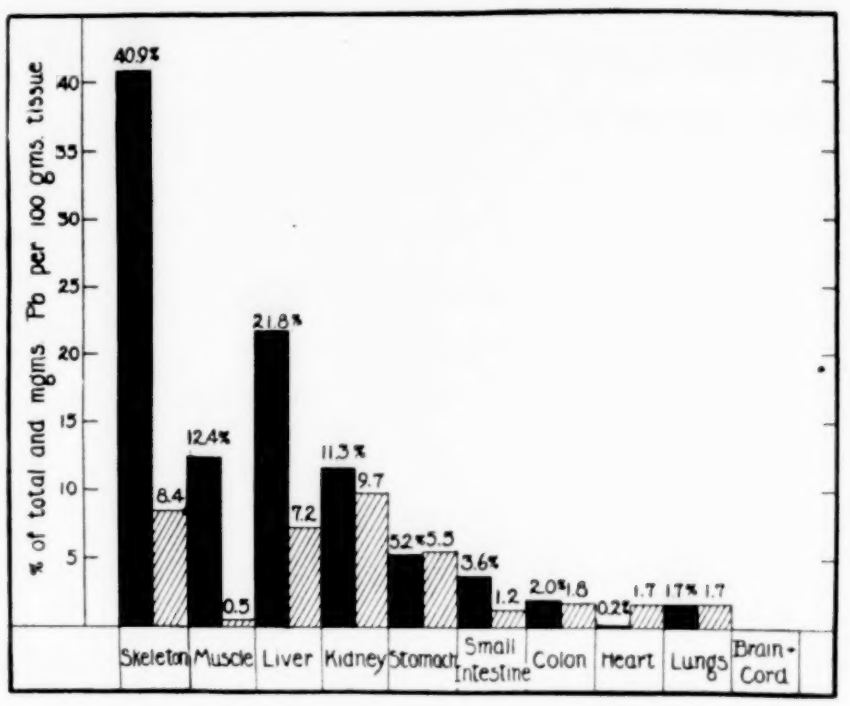


FIG. 1.—Diagrammatic representation of the distribution and concentrations of lead in the tissues of Cat D. Solid black portions represent percentages of total lead in body. Cross-hatched portions represent milligrams of lead per 100 gm. of fresh tissue.

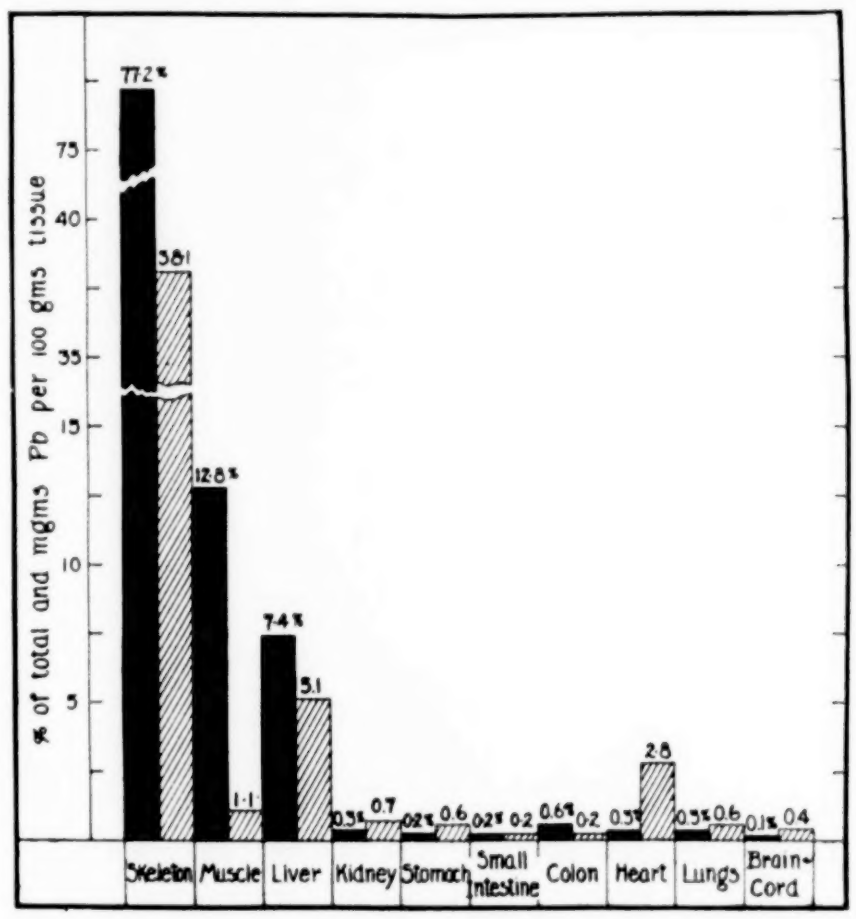


FIG. 2.—Diagrammatic representation of the distribution and concentrations of lead in the tissues of Cat J. Solid black portions represent percentages of total lead in body. Cross-hatched portions represent milligrams of lead per 100 gm. of fresh tissue.

imminent administration of lead was discontinued. With no treatment whatever, except good care, the cats gradually returned to normal condition; the weight lost was regained within a few weeks, and the animals seemed well in every way. Two cats from this group were killed and the tissues were analyzed; the rest were reserved for further experimental work which will be described in a later paper. Analytical data for these animals are presented in Table 3. Figure 3 shows diagrammatically the distribution of the lead stored in the body.

All the organs were analyzed as in earlier experiments, but lead was detected only in those tissues included in Table 3. Despite the interval of several weeks after ingestion of lead had ceased and despite the disappearance of all symptoms of plumbism, considerable amounts of lead were still retained in the body and were almost completely localized in the skeletal deposit. The innocuous effect of this stored lead as contrasted with the marked toxicity of the same lead during the gradual process of absorption and transportation gives weight to the theory of Straub (10) and Erlenmeyer (11) that damage is done to the organism not by stored lead but by small amounts of lead carried in solution in the blood stream.

TABLE 3.—DISTRIBUTION OF LEAD IN TISSUES OF CATS KILLED SEVERAL MONTHS AFTER LAST DOSES OF LEAD BY MOUTH

| CAT | TOTAL LEAD RECEIVED | INTERVAL SINCE LAST DOSE | TOTAL LEAD FOUND IN BODY | PERCENTAGE DISTRIBUTION OF TOTAL LEAD | | | |
|---|---|---|---|---|---|---|---|
| | | | | SKELETON | LIVER | KIDNEY | GASTRO-INTESTINAL TRACT |
| | *gm.* | *days* | *mg.* | % | % | % | % |
| L | 1.68 | 82 | 20.53 | 98.5 | 1.5 | 0.0 | 0.0 |
| M | 11.09 | 160 | 97.29 | 97.0 | 0.6 | 1.4 | 1.0 |

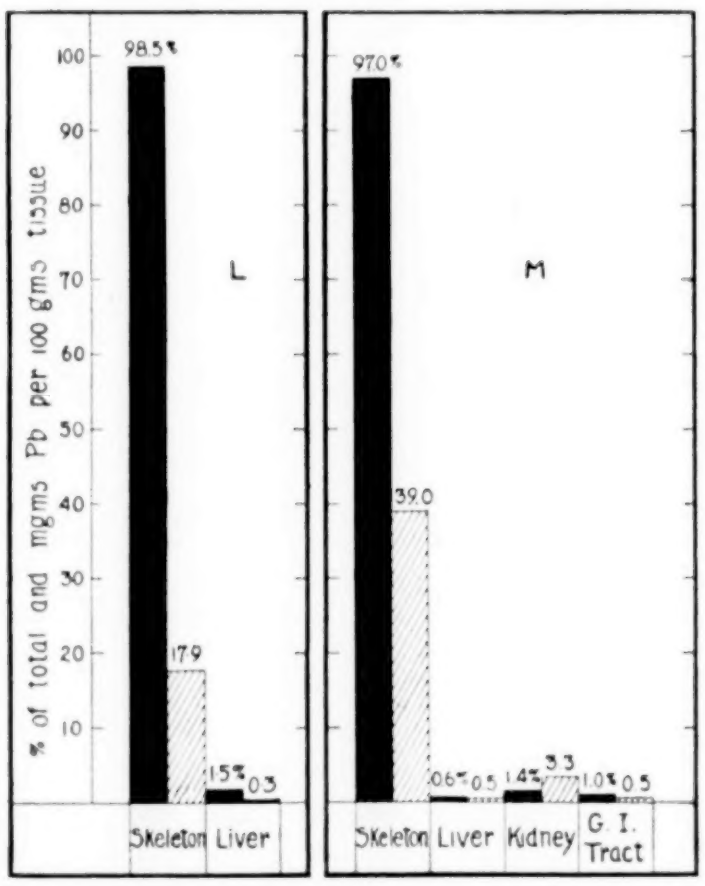


FIG. 3.—Diagrammatic representation of the distribution and concentrations of lead in the tissues of Cats L and M killed several months after the last dose of lead by mouth. Solid black portions represent percentages of total lead in body. Cross-hatched portions represent milligrams of lead per 100 gm. of fresh tissue.

The site of the deposition and storage of lead in the bones at once becomes of interest. Analytical data have been obtained from two sources. Separate analysis of marrow and solid bone of the larger bones of leaded animals, where separation is possible, has invariably shown a higher concentration in the solid portion.

In order to test further whether the marrow played any essential rôle in the deposition and storage of lead in the bones, hens were severely poisoned by

lead acetate. After death the pneumatic marrowless bones of the wings of four hens in different stages of poisoning were dissected out and analyzed. A range of from 10.7 to 83.9 mg. of lead per 100 gm. of tissue was found in them. These two groups of experiments indicate that lead is held in the solid portion of the bones, probably in the form of lead phosphate, since this is the most insoluble salt which could be formed in the body.

The suggestion made by Gusserow (1) regarding a possible migration of lead to and from the bony deposit should be recalled at this point. He believed that such a transfer might be analogous to that which exists for calcium and that it, likewise, is conditioned by changes in the organism as a whole. Should such a mobilization of stored lead occur, the "lead stream" theory of Straub and Erlenmeyer would emphasize the danger involved. In a later paper data on factors governing the migration of lead to and from the skeleton will be presented. Here, however, it is sufficient to point out that the storage of lead in the bones and the possibility of subsequent mobilization of redissolved lead are important clues to explanation of the latency and persistency of lead poisoning.

*Conclusions*

1. Lead, even in solution, when administered by mouth, is absorbed into the general circulation only in small amounts.

2. The liver serves as an effective protector for the rest of the organism by removing from the portal circulation and excreting in the bile much of the lead absorbed from the gastro-intestinal tract.

3. A small amount of lead reaches the general circulation and is distributed to the various tissues of the body.

4. The bones show special affinity for the lead in the blood stream and retain it. The concentration of lead in the other tissues does not increase as absorption continues. These two facts cause the relative as well as the absolute amount of lead in the skeleton to increase.

5. If absorption ceases and the subject returns to normal conditions, practically all the lead in the body becomes localized in the skeleton, where it is held as a temporarily harmless deposit.

6. The harm done by lead apparently occurs during the process of transportation rather than during storage in the body.

## BIBLIOGRAPHY


1. Gusserow, A.: Untersuchungen über Bleivergiftung. Arch. f. path. Anat., 1861, *21*, 443.
2. Rosenstein, S.: Ueber Epilepsia Saturnia und ihre Beziehung zur Urämie. *Ibid.*, 1867, *39*, 1, 174.
   Hitzig, E.: Studieren über Bleivergiftung. Berlin, 1868.
3. Heubel, E.: Pathogenese und Symptome der chronischen Bleivergiftung. Berlin, A. Hirschwald, 1871. Abstr. in Schmidt's Jahrb., 1871, *151*, 140.
4. Fairhall, L. T.: Lead Studies I: The Estimation of Minute Amounts of Lead in Biological Material. This Jour., 1922-1923, *4*, 9.
5. Ellenberger and Hofmeister: Zur physiologischen Wirkung und Deposition der Bleisalze bei Wiederkäuern. Ber. d. Veterinärw. in Königr. Sachs, 1883, Dresden, 1884, *28*, 102.
6. Prévost, G. L., and Binet, P.: Recherches expérimentales sur l'intoxication saturnine. Rev. méd. de la Suisse Rom., 1889, *9*, 606, 669.
7. Meillère, G.: Le saturnisme. Paris, Octave Doin, 1903.
8. Minot, A. S.: Lead Studies. II. A Critical

Note on the Electrolytic Determination of Lead in Biological Material. Jour. Biol. Chem., 1923, *55*, 1.

9. Kisskalt, K., and Friedmann, A.: Untersuchungen über Konstitution und Krankheitsdisposition. 2. Versuche über die Disposition zur Bleivergiftung. Ztschr. f. Hyg., 1914, *78*, 500.
10. Straub, W.: Ueber chronische Vergiftungen, speziell die chronische Bleivergiftung. Deutsch. med. Wchnschr., 1911, *37*, 1469.
    Straub, W.: Gift und Krankheit, nach Beobachtungen an experimenteller, chronischer Bleivergiftung. München. med. Wchnschr., 1914, *61*, 5.
11. Erlenmeyer, E.: Der Mechanismus der chronischen Bleivergiftung nach experimentellen Studien. Ztschr. f. exper. Path. u. Therap., 1913, *14*, 310.
    Erlenmeyer, E.: Experimentelle Studien über den Mechanismus der chronischen Bleivergiftung. München. med. Wchnschr., 1913, *60*, 1114.
    Erlenmeyer, E.: Nachweis und Bestimmung von Blei in organischem Material nebst einigen Bemerkungen über die Trennung von $PbSO_4$ und $CaSO_4$ durch Ammonacetat. Biochem. Ztschr., 1913, *56*, 330.
12. Carlson, A. J., and Woelfel, A.: The Solubility of White Lead in Human Gastric Juice, and its Bearing on the Hygiene of the Lead Industries. Am. Jour. Pub. Health, 1913, *3*, 755.
    Woelfel, A., and Carlson, A. J.: The Solubility of Lead Sulphide Ores and of Lead Sulphide in Human Gastric Juice. Jour. Pharmacol. and Exper. Therap., 1913-1914, *5*, 549.
13. Legge, T. M., and Goadby, K. W.: Lead Poisoning and Lead Absorption. London, Edward Arnold, and New York, Longmans, Green & Co., 1912, p. 62.
14. Meillère, G.: Localisation et élimination des poisons métalliques par les organes kératiniques dan les intoxications professionnelles. Compt. rend. Soc. de biol., 1902, *54*, 1134.
15. Brady, J. S.: Unpublished data.

# LEAD STUDIES V

## B. THE DISTRIBUTION OF LEAD IN THE ORGANISM AFTER ABSORPTION BY THE LUNGS AND SUBCUTANEOUS TISSUE*

A. S. Minot, Ph.D.

*From the Laboratories of Physiology of the Harvard Medical School*

### The Distribution of Lead in the Organism Following Absorption from the Lungs

#### *Introduction*

SINCE Stockhusen (1) established the causal relationship between lead and the disease prevalent among lead smelters, practically every investigator of the industrial lead hazard has agreed that the most dangerous trades are those which involve exposure to dust or to fumes arising from the molten metal. There is not, however, the same agreement in regard to the reason for this danger. Tanquerel des Planches (2) even as early as 1839 demonstrated by one experiment with insufflation of minium through a tracheal cannula in a dog that lead could be absorbed through the lungs. Comparatively recently Legge and Goadby (3) (4), with less conclusive experimental data, argued that lead is absorbed more rapidly by the lungs than by any other tissue; they therefore considered the inhalation of lead dust dangerous. Meillère (5), on the other hand, found very little lead in the lungs after death, and argued that the respiratory tract is, therefore, an unimportant portal of entry. He believed that finely divided dust is swallowed and that the toxic effects are produced by lead absorbed from the gastro-intestinal tract.

While workmen are exposed to lead dust in industry, absorption undoubtedly goes on simultaneously in both gastro-intestinal and respiratory tracts. The fate of lead particles in the respiratory tract can be properly studied only when other portals of entry are excluded. Blumgart (6), in this laboratory, has recently demonstrated the absorption of lead carbonate from the naso-pharynx of dogs so prepared that lead could enter neither the respiratory nor the gastro-intestinal tract.

#### *Description of Experiments*

In the present study of absorption of lead through the lungs the following standard preparation of the animal was adopted. With the animal under ether anesthesia, the neck was opened, aseptically, with as little trauma as possible, and the esophagus was securely ligated with four strong linen sutures below the level of the thyroid gland. The wound was then closed and generally required no further attention. While the animal was still anesthetized, a small glass tube was inserted, through the mouth, into the trachea nearly as far as the bifurcation. From 1 to 5 c.c. of a rather heavy suspension of a lead compound in sterile physiological salt solution ($p_H$7.4) were then introduced through this tube. Less postoperative lung infection has been found to follow the alternative method of injecting the lead suspension through a large needle inserted in the lumen of the exposed trachea. This is probably because bac-

*Received for publication May 12, 1924.

teria of the mouth are avoided. No leakage occurs, since the elasticity of the tissue completely closes the hole made by the needle. When the lead suspension was in the trachea, the cat's head was held high by elevating the catboard for a few minutes to facilitate insufflation of the particles by gravity.

To administer a quantitative dose by lung is practically impossible. Careful measurement of the amount of lead suspension introduced is useless because the animals invariably cough up an indefinite quantity; and this difficulty is even more unavoidable when lead dust is inhaled from the atmosphere.

In this series of experiments no attempt was made to determine the dose quantitatively; but gross, microscopic, and chemical examinations of the lungs in every case showed diffusely scattered particles of the lead compound present in the bronchioles and alveoli. Since the esophagus was closed—a fact always confirmed at autopsy—there was no possibility of absorption of lead from the gastro-intestinal tract. The duration of experiments was thus limited to the time that animals can be kept without food. Fluid, however, was introduced daily by intraperitoneal injections of 150 to 250 c.c. of sterile Ringer or 0.9 per cent. sodium chloride solution. The animals were well cared for in artificially warmed cages and showed no discomfort except that of fasting and the drooling caused by inability to swallow. Seven days was the maximum period of survival after operation.

Table 1 shows figures for the distribution of lead in the tissues of cats after insufflation of various solid lead compounds. Fifteen animals received lead as carbonate, two as chromate, three as sulphide, and five as oxide. Each group is arranged in order of duration of the experiments. This ranges from twelve to 162 hours.

All tissues were analyzed as in the feeding experiments (7), but determinable amounts of lead were detected only in the lungs, skeleton, liver, gastro-intestinal tract, and kidneys. Positive microchemical tests for lead by the method described by Fairhall (8) could, however, be obtained in most tissues. As the lead in the lungs was obviously an unabsorbed portion of the dose insufflated, it was not included in the distribution picture. The total quantities of lead found in the tissues exclusive of the lung vary from 2.6 to 72.4 mg. Except in extreme cases, there is no consistent ratio between the amount of lead absorbed and the duration of survival after insufflation. This indicates that factors such as the dispersion of particles in the lungs, the extent of local irritation produced, and the solubility of the compound inhaled, as well as massiveness of dose and duration of absorption, influence the rate at which lead enters the organism. In every instance, however, the amount of lead absorbed after a single insufflation of a suspended lead compound is in striking contrast to the much smaller quantities which would be absorbed in similar periods following the ingestion of larger doses of dissolved lead. Thus the quantity of lead present in the tissues twenty-four to forty-eight hours after administration by lung of 150 to 200 mg. of solid lead carbonate is as great as after the administration of several grams of lead in solution during weeks of continuous lead feeding. This fact emphasizes again the marked efficiency of the liver in preventing lead from entering the organism. When absorbed by the lung, lead at once enters the general circulation. Since only that small portion of blood carried in the hepatic

artery goes directly to the liver, efficient short circuiting of the lead in the portal and biliary circulations and liver is impossible. Instead all the absorbed lead is distributed by systemic blood.

This difference is demonstrated by figures showing the distribution of lead in the tissues. As has been noted in earlier experiments, there appears to be a marked tendency for the bones to remove and retain lead carried in the systemic blood. In the entire group of animals studied this skeletal deposit represents from 62.8 to 100 per cent. of the total measured quantity of lead exclusive of that remaining in the lungs. The most complete selective localization (generally between 90 and 100 per cent. of the total lead) in the skeleton is found as a rule in animals where the experiment lasted from twenty-four to seventy-two hours (see Fig. 1). This deposition of lead from the circulating blood is the same as that which occurs on a much smaller scale when lead escapes excretion through the liver after oral administration. The fact that only small amounts of lead reach the liver in these cases makes the distribution here resemble that observed when the liver has ceased to retain lead and selective localization in the skeleton has become marked in very advanced chronic lead poisoning after gastro-intestinal absorption.

The more general distribution of lead in animals which survive longer than about four days after operation is found so consistently as to be significant. Figure 2 shows diagrammatically the distribution in three animals living ninety hours or more after operation. The skeleton still retained a large percentage of the absorbed lead; but 12 to 30 per cent. of the total, in contrast to the small traces found in shorter experiments, appeared in the liver and the

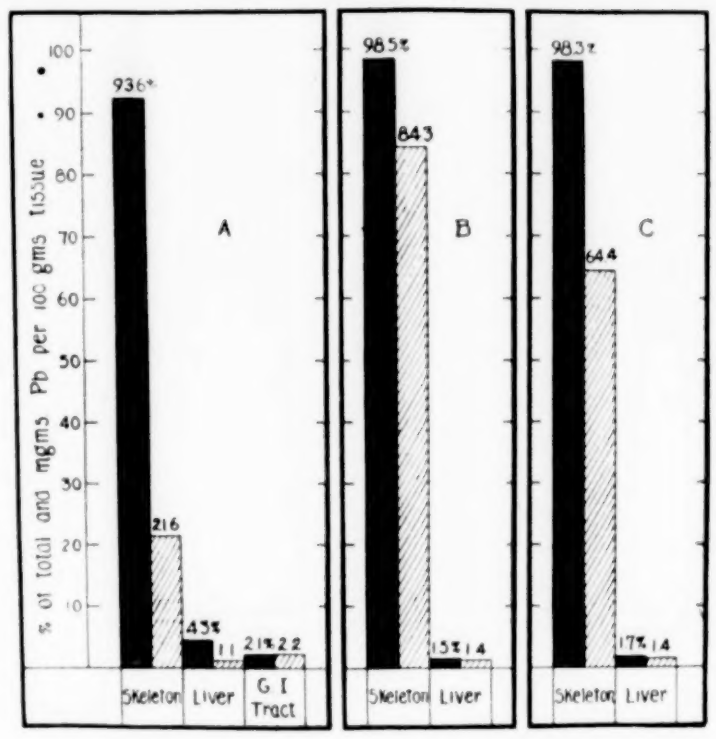


FIG. 1.—Diagrammatic representation of the distribution and concentrations of lead in Cats 2, 5, and 8. Solid black portions represent percentages of total lead in body exclusive of lungs. Cross-hatched portions represent milligrams of lead per 100 gm. of fresh tissue. No lead was detected in tissues not represented in the figure.

A. Cat 2, nineteen hours after insufflation of 5 c.c. of lead carbonate suspension. Total lead in body exclusive of lungs, 26.62 mg.

B. Cat 5, forty-two hours after insufflation of 4 c.c. of lead carbonate suspension. Total lead in body exclusive of lungs, 72.40 mg.

C. Cat 8, seventy-two hours after insufflation of 5 c.c. of lead carbonate suspension. Total lead in body exclusive of lungs, 71.87 mg.

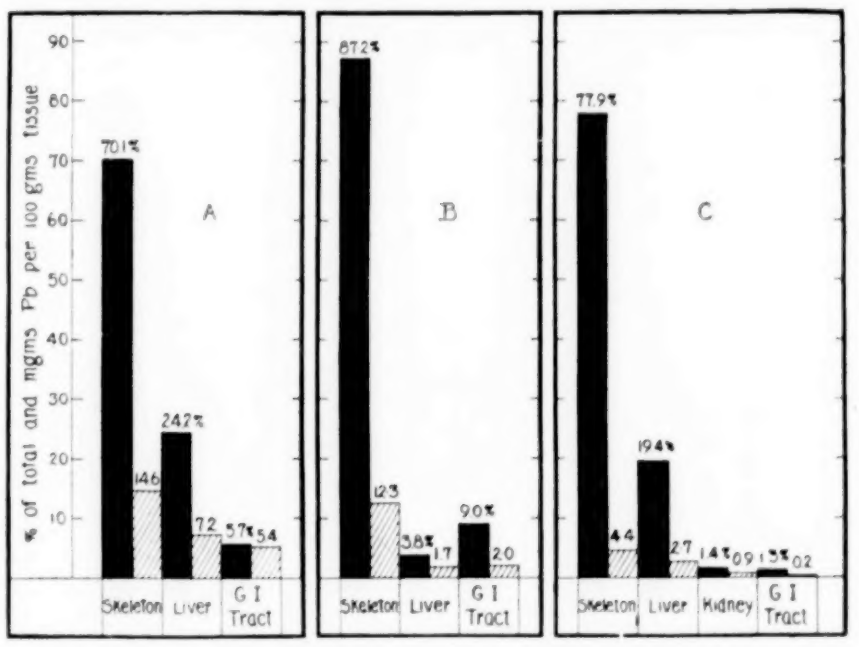


FIG. 2.—Diagrammatic representation of the distribution and concentrations of lead in the tissues of Cats 11, 15, and 13 ninety hours or more after insufflation of lead carbonate. Solid black portions represent percentages of total lead in body exclusive of lungs. Cross-hatched portions represent milligrams of lead per 100 gm. of fresh tissue. No lead was detected in organs not represented in the figure.

A. Cat 11, ninety hours after insufflation of 5 c.c. of lead carbonate suspension. Total lead in body exclusive of lungs, 35.08 mg.

B. Cat 15, 162 hours after insufflation of 5 c.c. of lead carbonate suspension. Total lead in body exclusive of lungs, 26.25 mg.

C. Cat 13, ninety-six hours after insufflation of 2 c.c. of lead carbonate suspension. Total lead in body exclusive of lungs, 12.71 mg.

TABLE 1.—DISTRIBUTION OF LEAD IN CATS WHICH HAVE RECEIVED SOLID LEAD COMPOUNDS BY LUNG, THE GASTRO-INTESTINAL TRACT BEING EXCLUDED AS A PORTAL OF ENTRY

| NUMBER | WEIGHT OF CAT | DURATION OF EXPERIMENT | AMOUNT OF SUSPENSION INSUFFLATED | TOTAL LEAD IN BODY | PERCENTAGE DISTRIBUTION OF TOTAL LEAD | | | | REMARKS |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | SKELETON | LIVER | GASTRO-INTESTINAL TRACT | KIDNEY | |
| | *kg.* | *hours* | *c.c.* | *mg.* | % | % | % | % | |
| *Experiments with Lead Carbonate Suspension*[1] | | | | | | | | | |
| 1 | 1.5 | 12 | 5.0 | 2.62 | 86.6 | 13.4 | trace | 0.0 | Poor condition. Bronchopneumonia. |
| 2 | 3.5 | 19 | 5.0 | 26.62 | 93.6 | 4.3 | 2.1 | 0.0 | Fair condition. Some congestion of lungs. 150 c.c. 0.9% NaCl solution given intraperitoneally. |
| 3 | 3.2 | 24 | 5.0 | 16.72 | 90.5 | 6.0 | 3.5 | 0.0 | Very good condition. 150 c.c. Ringer solution intraperitoneally. |
| 4 | 1.6 | 24 | 5.0 | 18.43 | 100.0 | 0.0 | 0.0 | 0.0 | Poor condition. Much respiratory disturbance. No fluid given. |
| 5 | 3.6 | 42 | 4.0 | 72.40 | 98.5 | 1.5 | 0.0 | 0.0 | Developed bronchopneumonia. No fluid given. |
| 6 | 3.5 | 48 | 5.0 | 16.22 | 96.1 | 3.9 | 0.0 | 0.0 | Many hemorrhagic areas in lungs. 100 c.c. Ringer solution intraperitoneally. |
| 7 | 2.0 | 56 | 5.0 | 14.75 | 92.0 | 8.0 | 0.0 | 0.0 | Lungs in fair condition. 75 c.c. Ringer solution and 100 c.c. 5% glucose solution intraperitoneally. |
| 8 | 3.0 | 72 | 5.0 | 71.87 | 98.3 | 1.7 | 0.0 | 0.0 | Lungs in good condition. 640 c.c. Ringer solution intraperitoneally. |
| 9 | 2.1 | 80 | 5.0 | 14.97 | 69.2 | 24.7 | 6.0 | 0.0 | Developed distemper. Right lung pneumonic. Left lung contained lead deposit. Otherwise normal. |
| 10 | 3.0 | 90 | 5.0 | 28.95 | 80.6 | 19.4 | 0.0 | 0.0 | Right lung showed marked congestion. Left lung fairly normal. 450 c.c. Ringer solution intraperitoneally. |
| 11 | 3.1 | 90 | 5.0 | 35.08 | 70.1 | 24.2 | 5.7 | 0.0 | Lungs fairly normal. 150 c.c. Ringer solution intraperitoneally. |
| 12 | 3.3 | 94 | 5.0 | 42.64 | 89.0 | 10.3 | 0.7 | 0.0 | Pneumonia developed on last day. 550 c.c. Ringer solution intraperitoneally. |
| 13 | 2.8 | 96 | 2.0 | 12.71 | 77.9 | 19.4 | 1.4 | 1.3 | Infection at site of wound. Lungs in good condition. 1,055 c.c. Ringer solution during experiment. Blood $CO_2$, 20.9 volume per cent. at death. |
| 14 | 3.1 | 120 | 1.5 | 26.32 | 93.9 | 4.1 | 1.4 | 0.8 | Lungs in very good condition. 950 c.c. Ringer solution during experiment. Blood $CO_2$, 30.0 volume per cent. at death. |
| 15 | 2.5 | 162 | 5.0 | 26.25 | 87.2 | 3.8 | 9.0 | 0.0 | Congestion of upper lobes of lungs. 940 c.c. Ringer solution during experiment. Blood $CO_2$, 19.2 volume per cent. at death. |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Experiments with Lead Chromate Suspension*[1] | | | | | | | | | |
| 16 | 2.3 | 60 | 2.0 | 17.11 | 100.0 | 0.0 | 0.0 | 0.0 | Slight bronchopneumonia. 200 c.c. Ringer solution intraperitoneally. |
| 17 | 1.8 | 100 | 2.0 | 16.51 | 97.5 | 3.3 | 0.0 | 0.0 | Lungs in bad condition—purulent. 400 c.c. Ringer solution intraperitoneally. |
| *Experiments with Natural Lead Sulphide (galena)*[1] | | | | | | | | | |
| 18 | 2.5 | 45 | 2.0 | 4.32 | 82.6 | 12.5[2] | 4.8 | ... | 150 c.c. 0.9% NaCl solution given intraperitoneally. Lungs somewhat congested. Died. |
| 19 | 3.2 | 70 | 2.0 | 25.77 | 94.0 | 3.8[2] | 1.8 | ... | 150 c.c. 0.9% NaCl solution given intraperitoneally. Marked emphysema due to tracheal puncture. Lungs heavily leaded. Very sick. Killed. |
| 20 | 3.0 | 98 | 2.0 | 14.36 | 97.2 | 1.5[2] | 1.2 | ... | 420 c.c. 0.9% NaCl solution given. Infected. Killed. |
| *Experiments with Lead Oxide (PbO)*[1] | | | | | | | | | |
| 21 | 2.3 | 24 | 2.0 | 2.95 | 62.8 | 14.0[2] | 23.4 | ... | 60 c.c. 0.9% NaCl solution given intraperitoneally. Poor recovery from ether. Some pneumonia. Very sick. Killed. Lungs congested. |
| 22 | 4.0 | 36 | 2.0 | 2.38 | 73.1 | 26.9[2] | 0.0 | ... | 175 c.c. 0.9% NaCl intraperitoneally. Lungs very badly congested. Very marked lead line. |
| 23 | 3.1 | 65 | 2.0 | 24.14 | 97.6 | 0.6[2] | 1.41 | ... | 300 c.c. 0.9% NaCl intraperitoneally. Lungs in good condition, not heavily "leaded." Died. Wound infected. |
| 24 | 4.0 | 96 | 2.0 | 4.29 | 84.6 | 6.1[2] | 9.3 | ... | 550 c.c. 0.9% NaCl intraperitoneally. Wound infected. Lungs quite normal. |
| 25 | 3.5 | 135 | 2.0 | 2.70 | 90.6 | 9.6[2] | 0.0 | ... | 700 c.c. 0.9% NaCl intraperitoneally. Lungs purulent and congested. Died. |

[1] In no case do percentage figures include in "total lead" that found in lungs.
[2] This figure includes both liver and kidney.

gastro-intestinal tract. Apparently some change in the organism as a whole hindered the deposition of lead in the skeleton so that more was free to reach the other tissues. That this was due simply to saturation of the skeleton with lead seems unlikely because of the extremely small amounts of lead present compared with the large mass of bony tissue.

A prediction as to the final explanation of this change in distribution would be premature at this point. But it is not out of place here to recall that the animals were fasting and receiving only fluid in the form of 0.9 per cent. sodium chloride solution. A depletion of the alkali reserve was shown by carbon dioxide determinations on the blood plasma. A distortion of the inorganic salt equilibria of the body fluids also probably existed. Unpublished work done in this laboratory has suggested the importance of increased acidity in hastening mobilization and excretion of stored lead. This would indicate that variations in both acid-base and inorganic salt equilibria may be fundamental in the explanation of the differences observed in these experiments and also in throwing light upon the general problem of storage and mobilization of lead in the organism.

The mechanism of absorption of lead by lung and of subsequent transportation to the skeleton and other tissues is not yet fully known. Microscopic examination of the lungs shows the lead compounds diffusely scattered through bronchioles and alveoli, many of the particles being contained in phagocytic cells, a condition similar to that described by Legge and Goadby (3, p. 23). Some of these cells are polymorphonuclear leukocytes but the majority are mononuclear endothelial cells—probably those which have been described by Permar (9) and recently discussed by Drinker (10).

Both chemical tests and the localization of lead in the body indicate that solution of the particles of lead compounds must occur during absorption. According to most authors, cells filled with insoluble particles taken from the lungs are eliminated either through the lymphatics or in the sputum. It is difficult to imagine how large numbers of such cells carried in the lymph could reach the skeleton where lead accumulates. The fact that after insufflation of lead chromate the lead deposit in the skeleton is free from any detectable trace of chromium—even when use is made of the extremely delicate diphenylcarbazide test for chromic acid described by Cazeneuve (11)—furnishes chemical evidence that solution and reprecipitation of the insufflated compounds must occur. This solution could be readily effected only at a hydrogen ion concentration considerably higher than that which generally is found in the body. Jacobs (12) has shown that it is possible, as a result of the penetration of carbonic acid, for a much higher degree of acidity to develop within cells than in the medium surrounding them. As a result of this it is conceivable that within the phagocytic cells conditions suitable for the solution of engulfed lead particles might be produced. Transportation of lead, either in true or colloidal solution, is probably effected through the blood stream. The skeleton removes and retains this lead, probably in the form of phosphate (13).

The pathological changes produced locally by such insufflation as has been described here are, of course, more marked than would usually be the case after gradual inhalation of the dust of a lead compound in industry. Follow-

ing the administration of smaller doses the lungs appeared grossly normal except that unabsorbed lead could be seen in patches of the same color as the compound introduced. These were localized, as a rule, in the lower part of the upper lobes, and in the upper part of the lower lobes. After larger doses, marked pulmonary consolidation often occurred, especially when the compounds had been administered by means of a tube entering the trachea through the mouth.

A comparison of the rate of absorption and of the distribution of the various lead compounds shows that the differences are quantitative rather than qualitative. The slow rate of absorption of lead oxide is unexpected because in industry the toxicity of lead oxide fumes is well known. The slight absorption in these experiments may be explained by the fact that insufflation of such doses of lead oxide caused very marked local congestion in the lungs which probably interfered with absorption by the blood stream. This artificial condition would, of course, not obtain when lead oxide particles were inhaled more gradually. In all the experiments the disposal of lead inhaled into the blood stream is similar.

This series of experiments conclusively proves what it was designed to demonstrate—namely, that lead is readily absorbed through the lungs. The abnormal condition of the animals, however, involved too many complicating factors to permit study of the type of poisoning which follows insufflation. The rate of absorption by the gastrointestinal tract is so slow compared with that by lung that even if small amounts of lead dust were coughed up and swallowed they would have comparatively little influence in the development of poisoning.

Another series of experiments, in which the esophagus was not ligated, was therefore carried out. The technic employed was as follows: Without cutting the skin, a needle was inserted into the trachea of an unanesthetized animal. The lead suspension was then introduced from a small syringe while the cat's head was elevated to facilitate inhalation of the lead. Doses of 1 to 2 c.c. of lead carbonate suspension (approximately 100 to 200 mg. of lead) were given. In some instances this injection was repeated at weekly intervals until three doses had been given.

Very little respiratory disturbance followed the injection, and until symptoms of chronic poisoning gradually developed, the animals seemed perfectly well. Definite lead lines appeared around the teeth within about a week, and there was a graduai decrease in appetite. Most of the animals lost much weight and strength. After only one dose of lead most of the cats eventually recovered, although toxic symptoms persisted for several weeks. If insufflation was repeated, however, the symptoms became more marked and were generally fatal; the animals died sooner than in ingestion experiments (7), but with practically the same symptoms.

Table 2 shows distribution figures for four untreated cases of poisoning due to insufflation of lead carbonate. Protocols 1 to 4 give the histories of the animals.

PROTOCOL 1.—Cat 222. Male, weight 3.9 kg.

*Feb. 2, 1923.* 2 c.c. of lead carbonate suspension injected into trachea, without anesthesia. Very little coughing.

*Feb. 7, 1923.* Erythrocyte count, 9,435,920 per cubic millimeter. No stippled red cells. Probably some polycythemia due to excitement caused by taking blood for count.

*Feb. 12, 1923.* Injection of 2 c.c. of lead carbonate suspension into trachea. Lead line on lower eye-tooth.

TABLE 2.—DISTRIBUTION OF LEAD IN CATS POISONED BY INSUFFLATION OF LEAD CARBONATE

| Number of Animal | Weight | | Number of Doses | Duration of Experiment | Total Lead in Body Exclusive of Lungs | Lead in Respiratory Tract at Death | Percentage Distribution of Absorbed Lead in Tissues | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Start | End | | | | | Skeleton | Muscle | Liver | Kidney | Washed Gastro-Intestinal Tract | Brain and Cord |
| | *kg.* | *kg.* | | *days* | *mg.* | *mg.* | % | % | % | % | % | % |
| 222 | 3.9 | 2.8 | 3 | 25 | 36.22 | 154.3 | 58.3 | 5.5 | 27.5 | 4.9 | 3.6 | 0.0 |
| 224 | 4.2 | 2.0 | 3 | 62 | 62.24 | 53.5 | 92.2 | 0.0 | 5.2 | 0.7 | 1.6 | 0.0 |
| 225 | 3.7 | 4.0 | 1 | 40 | 97.92 | 362.8 | 75.5 | 19.2 | 2.0 | 1.5 | 1.7 | ... |
| 226 | 3.6 | 2.6 | 1 | 42 | 60.49 | 123.1 | 78.6 | 10.4 | 7.3 | 2.6 | 0.7 | 0.4 |

*Feb. 14, 1923.* Weight 3.4 kg. Erythrocyte count, 9,912,000 per cubic millimeter. No typically stippled red cells, but a few polychromatophilic cells. Appetite poor.

*Feb. 21, 1923.* Weight 3.0 kg. Erythrocyte count, 10,399,640 per cubic millimeter. No stippled red cells. Appetite very poor. Injection of 2 c.c. of lead carbonate.

*Feb. 25, 1923.* Complete loss of equilibrium with a rotation of the head to the left and a tendency to fall toward the right. When placed on its feet, the cat would rush to the right side until it hit the wall or fell. No rigidity or paralysis. Apparently normal intelligence and consciousness of surroundings. During restlessness and excitement pupils widely dilated and hair on end.

*Feb. 27, 1923.* The symptoms more and more marked. Convulsions frequent. Death during a convulsion. Weight at death 2.8 kg.

Duration of experiment, 25 days.

PROTOCOL 2.—Cat 224. Male, weight 4.2 kg.

*Feb. 2, 1923.* Injection of 2 c.c. of lead carbonate suspension into trachea. Very little respiratory disturbance.

*Feb. 7, 1923.* Lead line around lower front teeth. Erythrocyte count, 10,812,800 per cubic millimeter. No stippled red cells.

*Feb. 12, 1923.* Injection of 2 c.c. of lead carbonate suspension. Considerable respiratory distress several hours after injection. Asthmatic attacks followed by periods of normal breathing.

*Feb. 14, 1923.* Weight 3.4 kg. Breathing gradually improved until normal. Erythrocyte count, 8,954,160 per cubic millimeter. No typical stippled red cells.

*Feb. 21, 1923.* Weight 2.8 kg. Injection of 2 c.c. of lead carbonate into trachea. No respiratory difficulty. Poor appetite.

*Feb. 28, 1923.* Weight 2.5 kg. Erythrocyte count, 7,590,480 per cubic millimeter. No stippled red cells. Appetite somewhat improved.

*March 7, 1923.* Weight 2.3 kg. Appetite fairly good. Progressive loss of weight and strength. Lead line very marked.

*March 27, 1923.* Weight 2.0 kg. Animal weak. Appetite fair. Erythrocyte count, 6,598,560 per cubic millimeter.

*April 4, 1923.* Appetite fair. Animal in poor condition. Weight 1.9 kg. Animal killed and tissues studied.

Duration of experiment, 62 days.

PROTOCOL 3.—Cat 225. Male, weight 3.7 kg.

*Nov. 5, 1923.* Injection of 2 c.c. of lead carbonate into lumen of trachea. No immediate reaction.

*Nov. 12, 1923.* Very marked lead line. Stomatitis.

*Dec. 13, 1923.* Appetite somewhat irregular.

*Dec. 15, 1923.* Animal in good health. Killed. Weight 4.0 kg.

Duration of experiment, 40 days.

PROTOCOL 4.—Cat 226. Female, weight 3.6 kg.

*Nov. 5, 1923.* Injection of 2 c.c. of lead carbonate suspension into lumen of trachea.

*Nov. 6-15, 1923.* Frequent vomiting. Teeth very sore and bleed easily. Lead line very marked. Appetite very poor.

*Dec. 3, 1923.* Weight 2.6 kg. Appetite poor. Condition of animal progressively worse. Animal weak.

*Dec. 15, 1923.* Cat very weak. Symptoms of distemper. Constipation.

*Dec. 16, 1923.* Cannot stand. Slight con-

vulsions. Administration of 0.8 gm. $CaCl_2$ in 400 c.c. Ringer intraperitoneally during day.

*Dec. 17, 1923.* Cannot stand alone. Killed. Weight 2.0 kg.

Duration of experiment, 42 days.

In postmortem examination it was found that the lungs of these animals were grossly normal, except for small areas where deposits of unabsorbed lead carbonate were visible. Since some of the lead given in these experiments was undoubtedly coughed up and swallowed, absorption must have taken place in both the lungs and the gastro-intestinal tract. In two animals (222 and 226) which died with very severe signs of poisoning and marked loss of appetite, the 20 to 40 per cent. of absorbed lead which remained outside the skeleton was widely distributed. Cat 225, although it was in good condition when killed, also showed this wide distribution, but in this case there may be a possible error due to the fact that much of the injected lead was found outside the trachea. As far as possible all of the visible deposit was removed before chemical examination, but contamination of the muscles of this region might account for the large quantity of lead found in the muscular tissue as a whole. Cat 224 was the subject of an experiment of longer duration. In spite of severe poisoning, it continued to eat well until death. When this animal was killed, it had received no lead for six weeks and the distribution of lead in the body was found to be similar to that characteristic of animals after absorption of lead has ceased.

### *Conclusions*

The two series of insufflation experiments just described seem to justify the following conclusions:

1. Lead is readily absorbed through the lungs after insufflation of finely divided solid particles. Since the various salts introduced are not found as such in the skeletal deposit, solution and reprecipitation of the lead must occur.

2. Because lead absorbed through the lungs enters the general circulation directly, it is more promptly distributed through the organism than that absorbed into the portal blood from the gastro-intestinal tract. This is because the liver is unable to remove and excrete most of this absorbed lead. The reason for the well-known danger of exposure to lead dust in industry is thus explained.

3. Whatever may be the path of entry, the skeleton always exercises a strong attraction for lead in the blood stream. After insufflation, since only small amounts of lead can reach the liver and be excreted in the bile, the skeletal deposit is relatively greater than after gastro-intestinal absorption of the same duration. In chronic cases, following the absorption of lead by either route, the distribution within the body is quite similar and shows an almost complete selective localization of lead in the bones.

## Distribution of Lead in the Organism Following Absorption from a Subcutaneous Deposit

Several investigators have administered lead subcutaneously in order to secure very slow and gradual absorption. Since, in industry, this type of exposure to lead is uncommon, such experiments are of little practical value. In this study, however, doses of 220 mg. of lead—sterile carbonate suspension—were injected subcutaneously into six cats. These animals were kept in metabolism cages, and though no attempt was made to obtain total recoveries, the lead excretion in urine and feces was

TABLE 3.—DISTRIBUTION OF LEAD IN CATS AFTER LEAD ABSORPTION FROM SUBCUTANEOUS TISSUE

| Number | Duration of Experiment | Weight of Animal | | Unabsorbed Lead | Total Lead in Body | Percentage Distribution of Total Lead | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | At Start | At Death | | | Skeleton | Muscle | Liver | Kidney | Gastro-Intestinal Tract | Brain and Cord |
| | *days* | *kg.* | *kg.* | *mg.* | *mg.* | % | % | % | % | % | % |
| 1 | 132 | 2.7 | 3.3 | 110.45 | 25.72 | 86.7 | 2.0 | 6.6 | 0.7 | 3.5 | 0.0 |
| 2 | 149 | 3.3 | 4.0 | 157.99 | 29.20 | 99.4 | 0.0 | 0.0 | 0.6 | 0.0 | 0.0 |

studied from time to time. Small amounts of lead (2 to 3 mg. per week) were constantly excreted in the feces, and occasional traces appeared in the urine.

After four months, during which the only indications of lead intoxication were definite lead lines in certain cases, two animals were killed and their tissues were analyzed. Table 3 shows the distribution figures. The total amounts of lead stored in the body other than the still unabsorbed deposit were 25.72 and 29.20 mg., respectively. Of this about 90 per cent. was in the skeleton; most of the remainder was in the liver and gastro-intestinal tract.

In these animals very slow absorption on the one hand and excretion or harmless storage of lead in the skeleton on the other apparently served to maintain such a low concentration of lead in the blood stream that no noticeable symptoms of plumbism developed during the course of the experiment. Since in the two cases studied the disposal of lead was the same as that which follows gradual entrance of lead into the general circulation, the remaining four animals of this group were reserved for further experimental study.

Absorption through the skin has often been suggested as one of the possible sources of danger in industrial exposure to lead. Siissmann (14) has shown experimentally that it is possible to induce very slow absorption of certain lead compounds through the intact skin. In our experiments the extremely slow absorption of lead carbonate placed directly in the subcutaneous tissue indicates that absorption through the unbroken skin must be extremely slight. This, together with the fact that in industry cutaneous exposure to lead is usually accompanied by respiratory or gastro-intestinal exposure, makes contact of the intact skin with lead compounds of very minor importance as a cause of poisoning.

## General Summary and Discussion of Papers A and B

These studies of the distribution of lead in the organism after absorption by the respiratory or gastro-intestinal tracts or by the subcutaneous tissue seem to justify the following conclusions, which are also in accord with conditions observed in postmortem studies of human material after chronic lead poisoning.[1]

### *Absorption*

Lead is absorbed by the gastro-intestinal mucosa, the entire respiratory tract, and subcutaneous tissue. When it enters by the gastro-intestinal route,

[1] See Minot, A. S. and Aub, J. C.: Lead Studies V. C. The Distribution of Lead in the Human Organism. THIS JOUR., 1924-1925, *6*, 149.

lead is transported in the portal blood to the liver. This organ protects the rest of the organism rather efficiently by removing most of the lead carried in the portal vein and by excreting a large part of it in the bile. This mechanism greatly retards actual absorption of lead into the general circulation after ingestion. The total amounts found in the various tissues even after long exposure are always low. Gradually, however, lead reaches the systemic circulation, is distributed throughout the organism and sooner or later causes lead intoxication.

Lead compounds insufflated as solid particles are absorbed directly into the general circulation. Thus lead is distributed throughout the organism much more rapidly when it enters through the respiratory tract than when the liver can exercise its protective function of removal and excretion during the process of absorption.

Lead likewise enters the general cirlation directly from the subcutaneous tissue, but the rate of absorption by this route is so slow as to be of very little practical importance.

*Distribution*

The avidity with which the skeleton picks up and retains lead from systemic blood is very marked. Although during active absorption lead can generally be detected in all tissues, selective localization in the bones is apparent from the first. After absorption ceases, the lead retained by the organism gradually becomes almost completely localized in the skeleton.

*Storage*

After absorption has ceased and symptoms of poisoning have disappeared, a considerable amount of lead may still be retained by the skeleton. Such a store of lead is temporarily harmless and may be held indefinitely. Its skeletal deposit is, however, held in such a position that its release would flood the organism with lead; for this reason, it must be considered as a potential source of future danger.

## BIBLIOGRAPHY


1. Stockhusen: De Lithargyii Fumo Noxio Morbifico ejusque Metallico Frequentiori Morbo Vulgo Dicto Hüttenkatze. Gaslar, 1656.
2. Tanquerel des Planches, L.: Traité des maladies de plomb ou saturnines. Paris, Ferra, 1839, vol. 1, p. 88.
3. Legge, T. M., and Goadby, K. W.: Lead Poisoning and Lead Absorption. London, Edward Arnold, and New York, Longmans, Green & Co., 1912.
4. Goadby, K.: A Note on Experimental Lead Poisoning. Jour. Hyg., 1909, *9*, 122.
5. Meillère, G.: Le saturnisme. Paris, Octave Doin, 1903.
6. Blumgart, H. L.: Lead Studies VI. Absorption of Lead by the Upper Respiratory Passages. THIS JOUR., 1923-1924, *5*, 153.
7. Minot, A. S.: Lead Studies V. A. The Distribution of Lead in the Organism after Absorption by the Gastro-Intestinal Tract. *Ibid.*, 1924-1925, *6*, 125.
8. Fairhall, L. T.: Lead Studies VIII. The Microchemical Detection of Lead. Jour. Biol. Chem., 1923, *57*, 455.
9. Permar, H. H.: An Experimental Study of the Mononuclear Phagocytes of the Lung. Jour. Med. Res., 1920, *42*, 9.
   Permar, H. H.: The Development of the Mononuclear Phagocytes of the Lung. *Ibid.*, 147.
   Permar, H. H.: The Migration and Fate of the Mononuclear Phagocytes of the Lung. *Ibid.*, 209.
10. Drinker, C. K.: Modern Views upon the Development of Lung Fibrosis. THIS JOUR., 1921-1922, *3*, 295.
11. Cazeneuve, P.: Sur la diphénylcarbazide réactif très sensible de quelques composés métalliques: cuivre, mercure, fer au maximum,

acide chromique. Bull. Soc. chim. de Par., 1900, *23*, 701.

12. Jacobs, M. H.: To What Extent Are the Physiological Effects of Carbon Dioxide Due to Hydrogen Ions? Am. Jour. Physiol., 1920, *51*, 321.
    Jacobs, M. H.: The Production of Intracellular Acidity by Neutral and Alkaline Solutions Containing Carbon Dioxide. *Ibid.*, 1920, *53*, 457.
13. Fairhall, L. T.: Lead Studies X. The Deposition of Lead Salts, with a Note on the Solubilities of Di-Lead Phosphate in Water at 25° C. and of Di-Lead and Tri-Lead Phosphates in Lactic Acid at 25° C. THIS JOUR., 1924-1925, *6*, 159.
14. Süssmann, P. O.: Beitrag zur Frage der Permeabilität der intakten Haut für Bleiverbindungen. München. med. Wchnschr., 1918, *65*, 1407.

# LEAD STUDIES V

## C. THE DISTRIBUTION OF LEAD IN THE HUMAN ORGANISM*

A. S. Minot, Ph.D., and J. C. Aub, M.D.
*From the Laboratories of Physiology of the Harvard Medical School*

### *Introduction*

THE large number of chemical data in the literature contributes little toward a better understanding of chronic plumbism because of the almost universal failure to study individual cases intensively. Although chemical analyses have supplemented most of the more modern investigations, in nearly every case only the one or two tissues obviously involved have been examined —the gastro-intestinal tract in cases of colic, the brain in encephalopathy, etc. —so that no complete picture of the distribution of lead in the organism at any given stage in the disease has been obtained. It is impractical in this paper to enumerate such chemical data from the analysis of isolated tissues, but it is important to mention a few of the more thorough examinations.

One of the earliest investigators to make extensive studies of the distribution of lead in the human organism was Devergie (1) who collaborated with Tanquerel des Planches (2) in studying industrial lead poisoning. Although his work is of historical interest because he analyzed most of the tissues in a large number of cases, the crudity of the analytical methods of his time limits the value of the data. Comparatively recently Meillère (3) has carried out the most extensive chemical investigations of human tissues. The range of concentration of lead which he found in the various tissues in great numbers of analyses is shown in Table 1. Unfortunately the method of presentation of results obscures much of the information which must lie in such a mass of data. If the individual distribution pictures could be compared or contrasted, much more could be learned in regard to the relation between chemical findings and the stage and type of plumbism. Meillère, himself, brings out points in his discussion which show that he evidently made such comparisons. He has noticed that the lead, which is widely distributed during the period of absorption, tends to be progressively more and more localized in the keratinous tissues, bones, and central nervous system after exposure to lead has ceased. Oliver (4) likewise has con-

TABLE 1.—CONCENTRATION OF LEAD IN HUMAN TISSUES (MEILLÈRE)

| TISSUE | CONCENTRATION OF LEAD |
|---|---|
| | *mg. per kg. fresh tissue* |
| Hair of axilla and pubis | 500 to 5,500 |
| Hair of head | 200 to 2,700 |
| Teeth | 60 to 180 |
| Liver | 18 to 90 |
| Spleen | 2 to 80 |
| Gray matter of brain | 16 to 60 |
| Kidney | 6 to 35 |
| Skin | 2 to 44 |
| Ribs and costal cartilage | 4 to 25 |
| Large blood vessels | 8 to 16 |
| Lungs | 5 to 12 |
| Pancreas | 5 to 8 |
| Cerebellum | 2 to 8 |
| Heart | 3 to 6 |
| Muscle | 2 to 4 |
| White matter of brain | 1 to 4 |
| Thyroid | 2 to 3 |

*Received for publication June 23, 1924.

tributed rather complete studies of lead distribution in a few cases.

The purposes of this paper are (*a*) to present the figures obtained in seven autopsies by chemical analysis of the tissues after industrial exposure to lead, and (*b*) to apply similar information derived from recent animal experiments (5) to the interpretation of the necessarily less complete data which may be obtained from human autopsy material.

### *Discussion of "Normal Lead"*

Before proper diagnostic significance may be assigned to the concentrations of lead in the tissues, it must be ascertained whether or not lead is normally present in the human body. As Chevreul (6) and Devergie (1) pointed out, the term "normal lead" is poorly chosen. Lead is neither an essential nor a constant constituent of any human tissue, but may be present accidentally if the customary activities and diet of an individual allow the entrance of lead compounds into the organism. There is, therefore, only a quantitative difference between so-called normal lead and that found after symptoms of plumbism have developed. It is, nevertheless, important to know what amounts of lead may be absorbed by persons living a normal life at a given time and locality, because such data help in the interpretation of the findings after industrial exposure and provide general information about public health conditions. Devergie in 1840 published the following figures for what he called "natural lead" present in human tissues. Although these figures are probably very inaccurate owing to the methods of analysis, they seem, however, of sufficient historical interest to be included here.

| *Source of Tissue* | *Lead Found Calculated as $PbSO_4$ gm. in total organ* |
|---|---|
| A new-born infant—intestinal tube | 0.001 |
| Child 8 years old—stomach | 0.004 |
| Child 4 years old—intestinal tube | 0.025 |
| Adult healthy woman— stomach | 0.020 |
| Adult healthy woman—intestines | 0.040 |
| Adult man—intestines | 0.031 |
| Adult man—gallbladder and bile | 0.003 |
| Sick woman—intestines | 0.010 |
| Sick woman—brain (1 lb.) | 0.006 |

Legrip (7) and Millon (8) at about the same period found lead generally present in the blood and tissues of normal individuals. All three of these authors agree that lead is present in the organism because of the accidental contamination of food, wine, and water with lead compounds. At a time when much less care was exercised to obtain pure food and drinking water than has been the case more recently, such conditions are not surprising. In 1903 Meillère (9) took up this problem and found that small traces of lead were frequently, but by no means universally, present in the tissues of persons who were not exposed to lead in industry and who showed no symptoms of poisoning. In such cases lead was found most frequently in the hair, nails, and bones, where it appeared to be stored as a harmless deposit.

Since recent experiments have shown that lead retained in an apparently normal individual is to be found almost exclusively in the skeleton, it seemed most reasonable to test for the so-called normal lead by analyzing specimens of bone. Table 2 shows the concentrations of lead found in twenty-six specimens of human bone obtained from cases with no symptoms of plumbism and no recent industrial exposure to lead. In seven cases lead was present. Of these, four cases had a definite history of exposure to lead at some previous time.

TABLE 2.—ANALYSIS OF NORMAL HUMAN BONES

| NO. | SOURCE OF SPECIMEN | WEIGHT OF SPECIMEN | LEAD FOUND | LEAD PER 100 GM. | SEX | AGE | BRIEF HISTORY |
|---|---|---|---|---|---|---|---|
| | | *gm.* | *mg.* | *mg.* | | | |
| 1 | Femur | 152 | 0.00 | 0.00 | Male | 60 | Tinsmith for 25 years. Leg amputated for diabetic gangrene. |
| 2 | Tibia | 210 | 0.00 | 0.00 | Female | 57 | Housework until 3 years before. Amputation of lower leg for arteriosclerotic gangrene. |
| 3 | Ribs | 51 | 0.00 | 0.00 | Female | 27 | Clerk. Rib resected for unilateral pulmonary tuberculosis. |
| 4 | Ribs | 75 | 0.00 | 0.00 | Male | 31 | Weaver in cotton mill for 3 years. Resection of ribs for chronic empyema. |
| 5 | Tibia Fibula Tarsus | 332 | 0.87 | 0.26 | Male | 11 | Traumatic amputation of leg. Only known exposure to lead was careless use of paint 2 months before. |
| 6 | Tibia Fibula Toe | 225 | 0.00 | 0.00 | Male | 63 | Weaver. Amputation for arteriosclerotic gangrene. |
| 7 | Lower leg | 350 | 0.00 | 0.00 | Male | 58 | Fireman. Amputation for osteomyelitis. |
| 8 | Vertebra | 60 | 0.00 | 0.00 | Male | 44 | Occupation unknown. Lobar pneumonia. |
| 9 | Tibia Fibula | 225 | 0.00 | 0.00 | Male | 15 | Stock clerk for 3 months. Amputation for sarcoma. |
| 10 | Vertebra | 63 | 0.74 | 1.18 | Male | 60 | Veterinary. Until 15 years before death had used drinking water from well contaminated with lead. |
| 11 | Tibia | 351 | 0.00 | 0.00 | Male | 69 | Night watchman for 6 months. Usual occupation unknown. Amputation of left leg for arteriosclerotic gangrene. |
| 12 | Tibia | 208 | 0.00 | 0.00 | Male | 50 | Tailor. Amputation of a painful stump for osteitis. |
| 13 | Tibia | 162 | 0.00 | 0.00 | Female | 66 | Housewife. Amputation of unhealed stump for diabetic gangrene. |
| 14 | Foot | 245 | 0.22 | 0.089 | Male | 38 | Brakeman on railroad for 12 years, except for occasional painting. Painter 7 years previous to present occupation. Traumatic amputation. |
| 15 | Tibia | 362 | 0.00 | 0.00 | Male | 75 | Occupation unknown. |
| 16 | Vertebra | 61 | 0.72 | 1.18 | Female | 58 | Demonstrator and saleswoman. Frequently painted pictures, furniture, and interior of her house. Syphilis of stomach and cirrhosis of liver. |
| 17 | Tibia Fibula Foot | 810 | 0.00 | 0.00 | Male | 62 | Mill worker. Amputation for arteriosclerotic gangrene. |
| 18 | Tibia | 205 | 0.00 | 0.00 | Male | 52 | Occupation unknown. Traumatic amputation. |
| 19 | Femur | 420 | 0.00 | 0.00 | Male | 58 | Coal heaver for 20 years. Amputation. |
| 20 | Radius and ulna | 245 | 0.00 | 0.00 | Male | 45 | Laborer. Traumatic amputation of forearm. |
| 21 | Femur | 235 | 0.00 | 0.00 | Male | 15 | School boy. Amputation for septicemia. |
| 22 | Tibia | 340 | 7.08 | 2.08 | Male | 60 | Treer in shoe factory for 30 years. No known exposure to lead. Amputation for diabetic gangrene. |
| 23 | Femur | 300 | 0.00 | 0.00 | Male | 64 | Mechanic for 15 years. Amputation for arteriosclerotic gangrene. |
| 24 | Femur | 217 | 0.00 | 0.00 | Male | 33 | Twelve years in rubber plant, spreader and curer of rubbers. Amputation for progressive thrombo-angiitis. |
| 25 | Tibia | 132 | 1.21 | 0.90 | Female | 67 | Housewife. No known exposure to lead. Diabetic gangrene. |
| 26 | Tibia | 335 | 5.62 | 1.68 | Female | 65 | Cook. Amputation for diabetic gangrene. Only known exposure to lead was use of lead water pipes for many years. |

Although the other three patients could recall no activity involving exposure to lead, such a hazard might well have been overlooked. Nineteen specimens of bone, however, contained no lead, despite the fact that they were taken chiefly from old persons who had had prolonged opportunity for exposure. These figures indicate that in this vicinity food, water supplies, and the usual activities of daily life cause no retention of lead in the body.

### *Analytical Results*

Analyses have been made of the various tissues of seven patients with known histories of exposure to lead. The analytical data and the brief histories are presented for each case separately in order to allow comparison of the various stages of intoxication.

### CASE 1

P. K., female, aged 2 years.

*History.* The patient has had one convulsion previous to her present illness. For one year she has gradually developed an illness characterized by increasing weakness and vomiting; she now vomits after each meal. She has marked constipation (five to seven days without bowel movement).

Tremor and lead line developed during her stay in the hospital. She had been gnawing paint from a bed for some time before admission and continued to have perverted appetite for paint and linen. She was discharged five months after admission.

*Physical Examination* showed nothing except a weak, undernourished child far below par mentally.

*Laboratory Findings* showed glycosuria but normal blood sugar. A normal glucose tolerance curve was obtained. The blood showed secondary anemia and marked stippling.

* * * * * *

*Second Admission.* Two months later.

*History.* For five days before admission to the hospital the child was listless and without appetite. The day before admission she became drowsy, sleeping most of day. She had had constant convulsions since morning. She died a few hours after admission to the hospital.

*Physical Examination* showed the child in tonic convulsive state. No Kernig was elicited but normal reflexes were exaggerated.

*Laboratory Findings* showed a trace of albumin, considerable sugar, acetone, and slight trace of diacetic acid in the urine.

Spinal puncture showed fluid under slightly increased pressure, slightly cloudy and yellowish. There were 70 cells per cubic millimeter. Globulin content was not increased (?). Wassermann test was negative.

*Autopsy Findings.* Tissues appeared grossly normal.

*Diagnosis.* Chronic lead poisoning with encephalopathy.

*Analysis of Tissues.* The results are given in the accompanying table.

*Discussion.* This is an acute case of lead poisoning produced by the swallowing of lead paint by a mentally defective child. The

| TISSUE | WEIGHT OF ENTIRE ORGAN | WEIGHT ANALYZED | LEAD FOUND | LEAD PER 100 GM. OF TISSUE | APPROXIMATE LEAD IN TOTAL TISSUE |
|---|---|---|---|---|---|
| | *gm.* | *gm.* | *mg.* | *mg.* | *mg.* |
| Liver | 450.0 | 359.0 | 18.36 | 5.11 | 22.99 |
| Kidney | 97.0 | 70.0 | 0.70 | 1.00 | 0.97 |
| Pancreas | 11.0 | 11.0 | 1.09 | 10.00 | 1.09 |
| Spleen | 72.0 | 52.0 | 0.56 | 1.07 | 0.77 |
| Stomach | 54.0 | 54.0 | 0.35 | 0.64 | 0.35 |
| Small intestine | 176.0 | 176.0 | 0.43 | 0.24 | 0.43 |
| Colon | 115.0 | 115.0 | 0.81 | 0.70 | 0.81 |
| Lung | 190.0 | 83.6 | 0.27 | 0.32 | 0.61 |
| Heart | 57.0 | 48.2 | 0.26 | 0.53 | 0.30 |
| Cerebrum | 745.0 | 745.0 | 2.91 | 0.39 | 2.91 |
| Cerebellum and medulla | 105.0 | 105.0 | 0.65 | 0.62 | 0.65 |
| Vertebrae | 1,280.0[1] | 13.0 | 1.99 | 15.30 | 195.80 |
| Bile | .... | 1.5 | trace | .... | .... |
| Blood | .... | 25.0 | trace | .... | .... |
| Bladder contents | .... | 17.0 | trace | .... | .... |

[1] Weight of skeleton calculated as 16 per cent. of body weight.

high concentration of lead in the liver indicates continued gastro-intestinal absorption, while the presence of lead in the blood stream and throughout the organism leaves no doubt of the active participation of lead in producing the clinical picture. The high concentration of lead in the pancreas in a case showing glycosuria is of interest but of course only a single observation. The condition of acidosis indicated by the presence of acetone and diacetic acid in the urine suggests the reason for the liberation of lead stored in the bones. We have here probably an organism overwhelmed by lead entering the blood stream both from the gastro-intestinal tract and from the skeletal deposit.

## CASE 2

A. S., male, married, aged 42 years.

*History.* The patient has been exposed to lead since a small child, by watching his father spray trees with lead arsenate. For twenty-seven years he has worked with the forestry department, transporting and using lead arsenate as a spray. In summer during the spraying season he has had severe lead colic. Treatment for double wrist drop was begun twelve years ago and was continued for three years. Despite this, the paralysis of the arms has continued, and in the last three years difficulty in walking has developed. Exposure to lead has been somewhat reduced during the last few years. For three weeks before entering the hospital the patient had increasing delirium, hallucinations, marked mental irritability, loss of concentration, and difficulty in speech. There was occasional incontinence of both urine and feces. He had a history of syphilis in early life. Death was probably caused by pneumonia.

*Physical Examination* showed complete paralysis of the extensor muscles of the fingers, wrists, and forearms on both sides. There was slight flexion of the right leg but not of the left. Both knee jerks and ankle jerks were absent. On the right leg, Babinski, Shattuck, Oppenheim, and Gordon reflexes were positive. Sensation was apparently normal. There was marked decubitus. No lead line was noted.

*Laboratory Findings.* The blood showed a red count of 4,000,000 cells per cubic millimeter. There was an occasional but very doubtful stippled cell. The Wassermann test was twice negative. The spinal fluid was clear, colorless, and under normal pressure, and contained three cells per cubic millimeter. The slightest possible trace of globulin was observed. There was increased sugar. The Wassermann test was positive. The gold test showed a tabetic curve.

*Autopsy Findings.* Autopsy showed fibrinous pleuritis, cystitis, atrophy of the extensor muscles of the forearm, arteriosclerosis, pachymeningitis and leptomeningitis of the cord, degeneration of ulnar and median nerves with fibrous tissue replacements, and lobar pneumonia of the right lower lobe.

*Diagnosis.* Pneumonia complicated by syphilis and chronic lead poisoning.

*Analysis of Tissues:*

| TISSUE | WEIGHT OF ENTIRE ORGAN | WEIGHT ANALYZED | LEAD FOUND | LEAD PER 100 GM. OF TISSUE | APPROXIMATE LEAD IN TOTAL TISSUE |
|---|---|---|---|---|---|
| | *gm.* | *gm.* | *mg.* | *mg.* | *mg.* |
| Liver | 1,555 | 1,390.0 | 0.22 | 0.01 | 0.23 |
| Kidney | 320 | 247.0 | 0.23 | 0.09 | 0.30 |
| Pancreas | 81 | 81.0 | 0.20 | 0.25 | 0.20 |
| Spleen | 79 | 79.0 | trace | .... | .... |
| Stomach | 122 | 122.0 | trace | .... | .... |
| Small intestine | 765 | 765.0 | 0.26 | 0.03 | 0.26 |
| Colon | 397 | 397.0 | trace | .... | .... |
| Lung | 1,346 | 1,346.0 | 0.17 | 0.01 | 0.17 |
| Heart | 290 | 219.0 | trace | .... | .... |
| Muscle | .... | 501.0 | 0.00 | 0.00 | 0.00 |
| Peripheral nerve, cord, and dura | .... | 25.0 | trace | .... | .... |
| Cortex and basal ganglia | 1,345 | 962.0 | 0.22 | 0.02 | 0.31 |
| Cerebellum | 117 | 117.0 | 0.20 | 0.17 | 0.20 |
| Gastro-intestinal contents | .... | 60.0[2] | 0.17 | 0.28 | .... |
| Vertebrae | 9,600[1] | 68.0 | 2.27 | 3.31 | 390.00 |
| Femur | | 42.7 | 2.04 | 4.77 | |
| Feces | .... | 15.0[2] | 0.22 | 1.47 | .... |
| Urine | .... | 2,500.0 | 0.00 | 0.00 | .... |

[1] Weight of skeleton calculated as 16 per cent. of body weight.

[2] Dry weight.

*Discussion.* This is a case of lead poisoning of long duration. The tissues were analyzed several months after the last exposure, and the wide distribution indicates that a mobilization of stored lead has occurred. This is associated with a severe terminal illness of a month's duration involving great emaciation, diminished intake of food, and a severe infection.

## CASE 3

E. B., male, single, aged 25 years.

*History.* There was no exposure to lead previous to the last seven months, during which the patient has worked in the mixing room of a rubber company. The room was very dirty and the workmen were exposed to much dust which contained lead compounds. The patient felt well until five months before death, when he began to have abdominal pains, marked constipation, and occasional attacks of dizziness. He has been out of work two weeks in the last five months on account of abdominal pains. Nine days before admission to the hospital, while changing his clothes in a factory in the morning, he suddenly became blind in his left eye and his left arm and leg were temporarily paralyzed. This attack passed off and he went to work. Nine days later he fell to the floor where he remained for a few minutes. Suddenly he got up and became so violent that five men were required to hold him. He was taken to a psychopathic hospital where clonic convulsions continued. Two days after admission he was given 800 c.c. of a 3 per cent. solution of sodium bicarbonate intravenously. He died fourteen hours later.

*Physical Examination.* The patient appeared to be in coma. The pupils reacted to light. All reflexes were normal except that both knee jerks were hyperactive. There was possibly a slight bilateral ankle clonus. The abdominal wall was somewhat sunken and was held rigid. There was a marked lead line. The patient had incontinence of urine and feces. The rectal temperature was 102°. The systolic blood pressure was 120, the diastolic 75.

*Laboratory Findings.* Urine examination revealed the slightest possible trace of albumin, three to five white blood cells in a microscopic field, and a few hyaline and granular casts. The blood findings were: hemoglobin 80 per cent., white blood count 28,000, red blood count 3,352,000, polymorphonuclear leukocytes 88 per cent., lymphocytes 9 per cent., large mononuclears 3 per cent. There was marked stippling. The Wassermann test was positive. The blood contained 54 mg. of non-protein nitrogen per 100 c.c. The spinal fluid was clear and contained 94 cells per cubic millimeter (nearly all were small lymphocytes). The albumin and globulin tests were ++, the colloidal gold and Wassermann tests negative.

*Autopsy Findings* showed a well-developed male negro. The gross pathological findings were: definite lead line, evidence of pulmonary edema and bronchopneumonia, and degeneration of the testicles. Grossly the brain appeared somewhat edematous, and the dura and pia were thickened. Microscopically the pia was seen to contain excessive fibrous connective tissue with many large mononuclear cells and lymphocytes, especially over the frontal, occipital, and cerebellar regions and in the cord. The arterioles were somewhat thickened and there was some degeneration of nerve cells of the frontal lobe.

*Diagnosis.* Lead encephalopathy and question of syphilis.

*Analysis of Tissues:*

| TISSUE | WEIGHT OF ENTIRE ORGAN | WEIGHT ANALYZED | LEAD FOUND | LEAD PER 100 GM. OF TISSUE | APPROXIMATE LEAD IN TOTAL TISSUE |
|---|---|---|---|---|---|
| | *gm.* | *gm.* | *mg.* | *mg.* | *mg.* |
| Liver | 1,380.0 | 609.6 | 0.77 | 0.12 | 1.65 |
| Kidney | 270.0 | 218.4 | 0.77 | 0.35 | 0.91 |
| Spleen | 140.0 | 129.0 | 2.08 | 1.59 | 2.23 |
| Lung | 1,520.0 | 932.0 | 2.75 | 0.29 | 4.41 |
| Heart | 340.0 | 225.0 | 0.71 | 0.32 | 0.99 |
| Ribs | 12,400.0[1] | 144.0 | 0.81 | 0.56 | 280.00 |
| Vertebrae | | 143.0 | 5.63 | 3.93 | |
| Cerebellum | 146.6 | 106.0 | 0.51 | 0.49 | 0.71 |
| Cerebrum | 1,173.4 | 705.0 | 0.00 | 0.00 | 0.00 |
| Blood | ..... | 180.0 | 0.50 | 0.27 | .... |
| Bile | ..... | 10.0 | 0.00 | 0.00 | .... |
| Intestinal contents | ..... | 50.0 | 0.90 | 1.80 | .... |

[1] Weight of skeleton calculated as 16 per cent. of body weight.

*Discussion.* This is a case of lead encephalopathy following exposure to lead dust until two days before death. The presence of lead in the blood stream and the general distribution, as well as the large amount of lead in the lungs, indicate that lead transportation and absorption were going on until death.

## CASE 4

J. G., male, married, aged 60 years.

*History.* The patient worked as a butcher all his adult life until six years ago. Since then he has worked regularly as a painter. He has remained perfectly well, has no history of colic, headaches, arthralgia, weakness, or pallor, and has never been constipated. Two days before death he fell down an elevator shaft while at his work. He was taken to the hospital in severe shock, where his treatment included the injection of 100 c.c. of 15 per cent. sodium chloride solution intravenously.

*Autopsy Findings.* Autopsy disclosed a fracture of the skull which followed the frontal sutures and extended into both middle ears. There was no evidence of lead poisoning.

*Diagnosis.* Fractured skull.

*Analysis of Tissues.* The results are given in the accompanying table.

*Discussion.* The sudden traumatic death in this case furnishes a picture of lead distribution which is probably typical of many lead workers without symptoms of plumbism. Significant amounts of lead are stored in the bone. The high concentration of lead in the liver indicates continued gastro-intestinal absorption, and in keeping with this the detection of lead in the kidney and the central nervous system shows lead in the process of transportation despite the absence of symptoms. The absence of lead in the lungs suggests that in this case there was little inhalation of lead dust.

| TISSUE | WEIGHT OF ENTIRE ORGAN | WEIGHT ANALYZED | LEAD FOUND | LEAD PER 100 GM. OF TISSUE | APPROXIMATE LEAD IN TOTAL TISSUE |
|---|---|---|---|---|---|
| | *gm.* | *gm.* | *mg.* | *mg.* | *mg.* |
| Liver | 1,600 | 1,150.0 | 7.84 | 0.68 | 10.88 |
| Kidney | 320 | 110.0 | 2.70 | 2.45 | 7.84 |
| Spleen | 75 | 74.5 | 0.00 | 0.00 | 0.00 |
| Lung | 1,390 | 583.0 | 0.00 | 0.00 | 0.00 |
| Cerebellum | 1,500 | 195.0 | 0.44 | 0.22 | 3.20 |
| Cerebrum | | 900.0 | 2.70 | 0.30 | |
| Vertebrae | 11,200[1] | wet 155.0 | 4.61 | wet 2.97 | 800.00[2] |
| | | dry 13.0 | | dry 35.46 | |
| Shaft of femur | | wet 23.4 | 2.56 | wet 11.36 | |
| | | dry 15.0 | | dry 17.00 | |

[1] Calculated by assuming 70 kg. as body weight and weight of skeleton as 16 per cent. of body weight.

[2] Estimated by taking average concentration for wet weights.

## CASE 5

C. S., male, married, aged 63 years.

*History.* For the last six years, until ten weeks before death, the patient has worked constantly as janitor for several tenements. He has done a good deal of painting, about which he was very careless. He has never shown symptoms of lead poisoning but has had gastric distress after periods of heavy drinking. For the last seven weeks he has had severe pain in the left chest with gradually increasing dyspnea, palpitation, and precordial pain on exertion.

*Physical Examination* showed auricular flutter and a pericardial friction rub. No lead line was observed.

*Laboratory Findings.* Blood examination showed 3,000,000 red cells per cubic millimeter. There was some achromia of the red cells, but no stippling.

*Autopsy Findings.* At autopsy an infarct of the wall of the left ventricle was found, as well as hypertrophy and dilatation of the heart. A mural thrombus was also present. Evidences of coronary and generalized arteriosclerosis, arteriosclerotic nephritis, and chronic passive congestion were observed.

*Diagnosis.* Infarct of the heart and auricular flutter.

*Analysis of Tissues.* The results are given in the accompanying table.

*Discussion.* This case was studied because of the history of exposure to lead. There were no signs or symptoms of plumbism present. The distribution shows nearly all of the lead stored in the bones.

| TISSUE | WEIGHT OF ENTIRE ORGAN | WEIGHT ANALYZED | LEAD FOUND | LEAD PER 100 GM. OF TISSUE | APPROXIMATE LEAD IN TOTAL TISSUE |
|---|---|---|---|---|---|
| | *gm.* | *gm.* | *mg.* | *mg.* | *mg.* |
| Liver | 1,505 | 240.0 | 0.31 | 0.13 | 1.95 |
| Kidney | 225 | 112.0 | 0.44 | 0.39 | 0.88 |
| Spleen | 265 | 185.0 | 0.00 | 0.00 | 0.00 |
| Lung | .... | 258.0 | 0.00 | 0.00 | 0.00 |
| Heart | 595 | 85.0 | 0.00 | 0.00 | 0.00 |
| Vertebrae | 9,600[1] | 160.0 | 11.85 | 7.40 | 897.00 |
| Femur | | 23.5 | 2.66 | 11.31 | |

[1]Weight of skeleton calculated as 16 per cent. of body weight.

## CASE 6

E. I., male, married, aged 51 years.

*History.* The patient has been a stereotyper for over twenty-five years, working seven hours a day in a small room where he stood over vats filled with molten metal (lead, antimony, and tin) from which fumes rose constantly. During the past nine months he has felt too weak to work, has had no appetite, and has become constipated. He has lost 50 pounds in weight. For six weeks he has been unable to swallow much food as it meets an obstruction. The patient was operated upon and gastrostomy performed seven days before death. The operation was followed by frequent feedings of milk and eggs.

*Physical Examination.* No lead line was observed. The marked lung pathology was explained by X-ray demonstration of an esophageal tumor with an esophago-tracheal fistula.

*Laboratory Findings.* The blood showed no anemia, stippling, or polychromatophilia.

*Autopsy Findings.* Autopsy disclosed a large carcinoma of the esophagus with two sinuses leading into the trachea and left lung; chronic pleuritis; atelectasis of the left lung; gastrostomy; and localized peritonitis.

*Diagnosis.* Carcinoma of the esophagus with esophago-tracheal fistula.

*Analysis of Tissues.* The results are given in the accompanying table.

*Discussion.* The detection of lead only in the skeleton simply indicates that lead has been absorbed during the previous exposure. The forced feeding with milk and eggs the last few days of life is probably partly responsible for the limited distribution.

| TISSUE | WEIGHT OF ENTIRE ORGAN | WEIGHT ANALYZED | LEAD FOUND | LEAD PER 100 GM. OF TISSUE | APPROXIMATE LEAD IN TOTAL TISSUE |
|---|---|---|---|---|---|
| | *gm.* | *gm.* | *mg.* | *mg.* | *mg.* |
| Liver | 1,435 | 740.0 | 0.00 | 0.00 | 0.00 |
| Kidney | 270 | 112.0 | 0.00 | 0.00 | 0.00 |
| Spleen | 60 | 32.0 | 0.00 | 0.00 | 0.00 |
| Heart | 205 | 92.0 | 0.00 | 0.00 | 0.00 |
| Pancreas | .... | 20.0 | 0.00 | 0.00 | 0.00 |
| Gastro-intestinal tract | .... | 276.0 | 0.00 | 0.00 | 0.00 |
| Vertebrae | 11,200[1] | 110.0 | 2.41 | 2.17 | 243.00[2] |
| Femur | | 12.6 | trace | .... | |

[1] Weight of skeleton calculated as 16 per cent. of body weight.
[2] Concentration of vertebrae assumed for entire skeleton.

## CASE 7

J. L., male, married, aged 53 years.

*History.* The patient had been a paint mixer for at least fifteen years. He worked generally with paints already mixed in oil and was therefore exposed to very little lead dust. Nine months before death he complained of abdominal cramps, lost weight, and showed pallor. He worked only two months after this.

*Physical Examination.* A consultant found a faint lead line nine months before death, but no other signs of lead. On the basis of the blood picture a diagnosis of pernicious anemia was made.

*Laboratory Findings.* The blood showed a red count of 1,200,000 per cubic millimeter, 40 per cent. hemoglobin, and no stippling. There was marked achromia and poikylocy-

tosis with moderate anisocytosis and a tendency to macrocytosis and polychromasia.

*Autopsy Findings.* At autopsy a lead line was found on the gums over several teeth. There were evidences of obliterative pericarditis and chronic nephritis. The bone marrow was light brick red. The brain was edematous.

*Final Diagnosis.* Cardiovascular and renal disease with anemia.

*Analysis of Tissues:*

*Discussion.* This patient was mildly exposed to lead for many years previous to seven months before death. Death was probably due to pernicious anemia. The absorbed lead was stored solely in the bones, indicating that absorption had ceased and that there was no mobilization of lead prior to death. The high concentration found in the bone marrow is probably due to the large amount of bone spicules which were present.

| TISSUE | WEIGHT OF ENTIRE ORGAN | WEIGHT ANALYZED | LEAD FOUND | LEAD PER 100 GM. OF TISSUE | APPROXIMATE LEAD IN TOTAL TISSUE |
|---|---|---|---|---|---|
| | *gm.* | *gm.* | *mg.* | *mg.* | *mg.* |
| Liver | 1,740 | 1,458.0 | 0.00 | 0.00 | 0.0 |
| Kidney | 245 | 219.3 | 0.00 | 0.00 | 0.0 |
| Spleen | 230 | 230.0 | 0.00 | 0.00 | 0.0 |
| Heart | 470 | 412.5 | 0.00 | 0.00 | 0.0 |
| Cerebrum | 1,510 | 1,076.0 | 0.00 | 0.00 | 0.0 |
| Cerebellum | | 180.0 | 0.00 | 0.00 | |
| Vertebrae | 8,000[1] | 50.0 | 0.81 | 1.62 | 195.6 |
| Femur | | 150.0 | 4.98 | 3.32 | |
| Marrow and spicules from femur | .... | 3.0 | 0.62 | 20.61 | .... |

[1] Weight of skeleton calculated as 16 per cent. of body weight.

## *General Discussion*

Animal experiments have demonstrated that during active absorption or following the mobilization of a skeletal store, lead can be detected in practically every tissue of the body. From the first, however, the marked tendency toward selective localization of the absorbed lead in the bones is apparent and becomes more and more evident as time elapses, particularly if exposure to lead has ceased.

A study of the relationship of analytical findings to clinical histories shows the same course of events in man. The distribution of lead in Case 1 is typical of that following gastro-intestinal absorption which continues until shortly before death. Evidence of this may be found in the clinical history and also in the analytical data, which show a high concentration of lead in the liver. Thus, in man as well as in animals the liver removes and retains much of the lead carried in the portal blood. Of the lead which escapes the liver and enters the general circulation a high percentage is retained by the skeleton, but while absorption continues lead is also present in the blood stream and in all the tissues of the body. In Cases 2 and 3 also lead was well distributed throughout the organism. Case 2 had had no exposure to lead for over six months—a fact corroborated by the observation that there was no marked accumulation in the lung or the liver. It therefore seems likely that the lead was mobilized from a skeletal store. In Case 3, on the contrary, relatively high concentration of lead in the lungs as compared with that in the liver clearly indicated that active absorption was taking place through the respiratory tract. This deduction was corroborated by the history of recent work at a very dusty lead trade. Further evidences that absorption was recent are the general distribution of lead throughout the body and the high concentration in the blood. In marked contrast to these cases are Cases 6 and 7 in which lead was found only in the skeleton. Ab-

sorption apparently had not taken place for some time and the stored lead had not been mobilized. Cases like these show conditions completely analogous to those observed in animals which have been killed so long after exposure that a complete selective localization of lead in the bones has taken place.

Since the diagnosis of lead poisoning is frequently of medicolegal as well as of clinical importance, it is well to consider what conclusions may be drawn from the distribution pictures obtained from cases with unknown or disputed histories. When lead is found exclusively in the bones, absorption must have occurred some time previously, but there is no justification for concluding that lead was involved as an acute cause of death. Similarly the finding of lead only in the feces and the gastro-intestinal tract may merely indicate that lead has been swallowed, and does not prove absorption. On the other hand, the presence of lead in the urine or in a majority of the body tissues demonstrates active transportation of lead by the blood and signifies either that absorption has recently occurred or that considerable quantities of lead have just been liberated from the bones. In either case danger has been incurred by bathing the tissues of the body with soluble lead, and lead may then rightfully be assumed to have been at least a complicating factor in the pathological picture.

### *Summary and Conclusions*

In actual cases of chronic plumbism in man events have been seen to run the same course as in experimental animals. While symptoms of plumbism are present, as a result either of absorption of lead from external sources or a mobilization of a skeletal store, lead is distributed generally throughout the organism. After exposure has ceased and lead is no longer entering the circulation there is almost complete selective localization of lead in a temporarily harmless skeletal deposit. The medicolegal importance of these data is discussed.

The pathological material for this study was obtained from various sources. Dr. George Burgess Magrath particularly, and also various officers of the Boston City, Children's, Massachusetts General, and Psychopathic Hospitals were very co-operative. It was their interest which made this collection of data possible.

### BIBLIOGRAPHY

1. Devergie: Quoted by Tanquerel des Planches. See Dana's translation, Lead Diseases: A Treatise from the French of L. Tanquerel des Planches. Boston, Tappan, Whittemore, and Mason, 1850, p. 352.
2. Tanquerel des Planches, L.: Traité des maladies de plomb ou saturnines. Paris, Ferra, 1839.
3. Meillère, G.: Le saturnisme. Paris, Octave Doin, 1903.
4. Oliver, T.: Lead Poisoning. London, H. K. Lewis, 1914, p. 118.
5. Minot, A. S.: Lead Studies V. A. Distribution of Lead in the Organism after Absorption by the Gastro-Intestinal Tract.
   B. Distribution of Lead in the Organism after Absorption by the Lungs and Subcutaneous Tissue. THIS JOUR., 1924-1925, *6*, 125, 137.
6. Chevreul: Quoted in Dana's translation (1), p. 351.
7. Legrip, V.: *Ibid.*, p. 353.
8. Millon, M.: *Ibid.*, p. 354.
9. Meillère, G.: Sur la présence normale du plomb dans l'organisme. Compt. rend. Soc. de biol., 1903, *55*, 517.

# LEAD STUDIES X

## THE DEPOSITION OF LEAD SALTS, WITH A NOTE ON THE SOLUBILITIES OF DI-LEAD PHOSPHATE IN WATER AT 25° C. AND OF DI-LEAD AND TRI-LEAD PHOSPHATES IN LACTIC ACID AT 25° C.*

LAWRENCE T. FAIRHALL, A.M., PH.D., AND CHARLOTTE P. SHAW, B.S.
*From the Laboratories of Physiology of the Harvard Medical School*

THE fact that bone tissue serves as a storehouse for whatever lead enters the circulation (1) is an indication that lead and calcium are deposited under much the same conditions. The qualitative evidence secured so far indicates that lead is present in the bones as phosphate; because of the insolubility of this salt its deposition with the calcium is not surprising. The early experiments of König (2), Lehnerdt (3), and Stoeltzner (4) on the substitution of strontium for calcium in the skeleton show quite clearly that other elements with somewhat similar properties may be substituted for calcium. If lead is deposited otherwise than as phosphate, it has not been possible as yet to demonstrate its presence as a more soluble salt, *e.g.*, carbonate. On the other hand, calcium phosphate is more soluble than calcium carbonate, but the proportion of calcium phosphate to carbonate in bones is approximately 87.5 : 12.5.

Lead, if present in the bone tissue as carbonate or albuminate, would be dissolved by dilute acetic acid. But acetic acid is not sufficiently strong to dissolve lead phosphate, and it is only by contact with nitric acid (in which lead phosphate is soluble) that lead may be completely removed from the skeleton. To determine the exact nature of the lead compound in the bones is very difficult, since the quantities of calcium phosphate and carbonate present are enormously greater than the amounts of lead. Indeed, it is difficult to ascertain the exact ratio of calcium carbonate to calcium phosphate in bone (5). Any chemical variations must inevitably affect the equilibrium relations of the lead and calcium compounds. The only practical solution of the problem, therefore, lies in identification of the lead compound *in situ*.

All qualitative evidence which has been obtained points to the conclusion that lead is present as phosphate. For instance, two cats with experimental lead poisoning were killed and their bones were examined for lead. Before the final chemical tests were made, the bones were scraped free of all adhering muscle tissue, dried, pulverized, and repeatedly washed with ether to remove fat. This clean bone powder (232 gm.) was then shaken with a large excess of 0.1N acetic acid for from twenty-four to forty-eight hours in a thermostat at 25° C. By this procedure only a trace of lead was extracted. After rewashing, tartaric acid (in which lead phosphate is soluble) was added, and 1.03 mg. of lead were removed—probably by surface action, for this acid cannot penetrate far into the bone particles because of the formation of insoluble calcium tartrate. Further extraction with 7 per cent. hydrochloric acid, which contained tartaric acid formed from the dissolved calcium tartrate, removed 14.43 mg. of lead. A

*Received for publication May 12, 1924.

second sample of dry, fat-free bone, weighing 120 gm., was treated with 0.1N hydrochloric acid for two hours. Sixty-five hundredths of a milligram of lead was extracted by this means. Further treatment for the same length of time with 0.1N acid (0.05N with tartaric and 0.05N with hydrochloric acid) removed 0.78 mg. of lead. The negligible amount dissolved by acetic acid and the relatively greater amount dissolved by tartaric than by hydrochloric acid would indicate the presence of phosphate rather than albuminate or carbonate.

The solubility of lead phosphate in water is 0.13 mg. per liter, while that of lead carbonate is 1.7 mg. per liter. Doubtless, when circulating lead comes into contact with the organic structure of the bones, it is deposited in much the same manner as the calcium salts, except that the competition of the phosphate group for lead must be much greater than for calcium.

Lead when precipitated *in equilibrio* as phosphate by di-sodium hydrogen phosphate yields di-lead phosphate. When first precipitated, however, tri-lead phosphate is obtained (6) and in the state of slight alkalinity that exists in the body the tri-lead salt is the stable form. This very insoluble salt is stored away in the bones and under normal conditions may remain inactive for comparatively long periods of time. It is transformed slowly in neutral or slightly acid solution into the di-lead salt. For this reason, the properties of the di-lead salt are of interest.

## The Solubility of Di-Lead Phosphate

Di-lead phosphate, prepared under equilibrium conditions, consists of a glittering mass of rhombic crystals. These proved to be more soluble than normal phosphate. This difference lends interest to the solubility of di-lead phosphate itself. Since solubility data for this salt are lacking, determinations were made directly. Sealed bottles containing a suspension of the pure salt in water were rotated end over end for twenty-four hours in an electrically heated thermostat at 25° C. After the solid phase had settled out on standing, filtered portions were drawn into a calibrated pipette and analyzed. Two different samples of salt were used, one of which was prepared by a synthetic method (7), the other (Series II) under equilibrium conditions. The results are given in Table 1.

TABLE 1.—SOLUBILITY OF DI-LEAD PHOSPHATE IN WATER AT 25° C.

| SERIES I | | SERIES II | |
|---|---|---|---|
| Gm. per Liter | Deviation from Mean | Gm. per Liter | Deviation from Mean |
| 0.0131 | 0.0005 | 0.0132 | 0.0003 |
| 0.0120 | 0.0009 | 0.0120 | 0.0009 |
| 0.0129 | 0.0000 | 0.0143 | 0.0014 |
| 0.0130 | 0.0001 | 0.0121 | 0.0005 |
| 0.0132 | 0.0003 | 0.0125 | 0.0001 |
| ..... | ..... | 0.0133 | 0.0004 |
| *Average* | | *Average* | |
| 0.0129 | 0.0004 | 0.0129 | 0.0007 |

The average value obtained (0.0129± 0.0005 gm. per liter) is approximately 100 times that for tri-lead phosphate. This pronounced difference is important, for any variation in local reaction, such as increased acidity, must change markedly the quantity of lead in circulation. Even as weak an acid as carbonic can react with tri-lead phosphate in the following manner:

$$Pb_3(PO_4)_2 + H_2CO_3 \rightleftharpoons PbCO_3 + 2PbHPO_4$$

Experiments were performed which corroborate this. Tri-lead phosphate was shaken for several hours in water saturated with carbonic acid at room temperature. The solution phase contained as much as 13.0 mg. of lead phos-

phate per liter. This corresponds to the solubility of di-lead phosphate in pure water.

A somewhat similar reaction occurs when tri-lead phosphate is shaken with lactic acid. In this case the phosphate neutralizes the lactic acid, and since lead lactate is soluble, there is much more lead in solution than is the case with carbonic acid.

$$Pb_3(PO_4)_2 + 2CH_3\text{-}CHOH\text{-}COOH \rightleftharpoons Pb(C_3H_5O_3)_2 + 2PbHPO_4$$

This difference in solubility is shown in Table 2 and also in Figure 1.

TABLE 2.—SOLUBILITY OF LEAD IN LACTIC ACID AT 25° C.

| NUMBER | LACTIC ACID NORMALITY | DISSOLVED DI-LEAD PHOSPHATE | DISSOLVED TRI-LEAD PHOSPHATE |
|---|---|---|---|
| | | *gm. per liter* | *gm. per liter* |
| 1 | 0.047 | 0.141 | 0.686 |
| 2 | 0.093 | 0.197 | 1.175 |
| 3 | 0.186 | 0.283 | 1.794 |
| 4 | 0.465 | 0.458 | 2.296 |
| 5 | 0.930 | 0.484 | 2.661 |

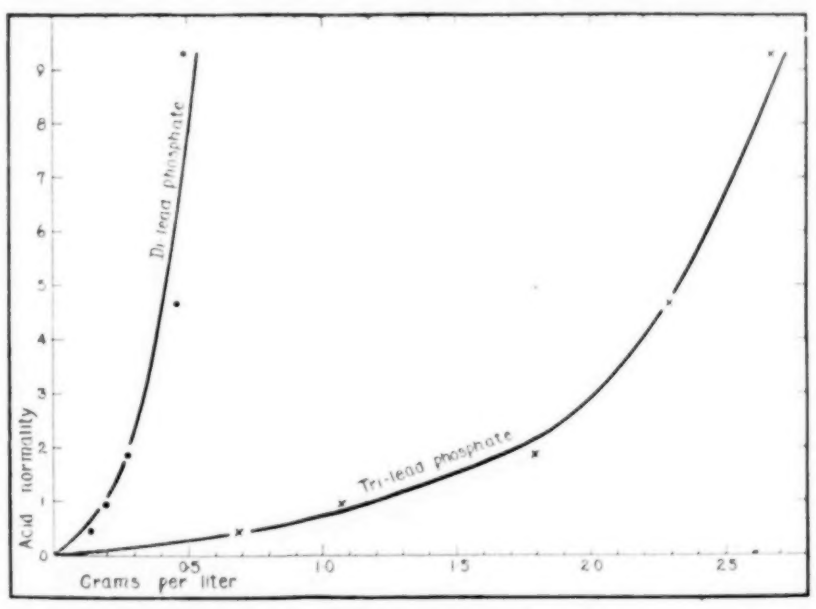


FIG. 1.—Solubility of lead phosphates in lactic acid at 25° C.

The failure of weak acids to dissolve lead *in vitro* from the bones of animals poisoned with lead is undoubtedly dependent in part upon the buffer action of calcium phosphate and in part upon the decreased solubility of lead phosphate in the presence of excess phosphate ions; but acids such as tartaric or nitric, with a specific solvent action on lead phosphate, may remove lead from bone tissue *in vitro*. It is, therefore, justifiable to conclude that changes in local reaction of the tissues are among the factors determining the quantity of lead that can pass into circulation. The solubility of tri-lead phosphate in lactic acid is particularly striking and suggestive; for lead phosphate, though insoluble under normal conditions, is sensitive to slight changes in acidity.

## THE ABSORPTION OF LEAD BY BONE

The object of the experiments detailed below is to reproduce a cycle of deposition and solution of lead such as may occur *in vivo*. Since lead is deposited in the skeleton, it seems pertinent to determine what happens when it comes in contact *in vitro* with bone tissue. Early experiments indicated that bones taken from animals immediately after death completely remove lead from aqueous or normal saline solutions. The usual procedure was to clean the bones carefully, remove the marrow, and shake the pulverized bone with lead chloride solution for a short time. The particles of bone were then well washed and analyzed for lead. These early experiments demonstrated that lead is completely and rapidly removed from dilute solutions of lead chloride and that contact with the bony substance itself rather than with the protein material is of chief importance in the absorption of lead.

Further experiments with a good grade of bone meal (carefully freed from fat with ether and as far as possible from protein by repeated extractions with salt solution and water) indicated that a much greater degree of absorption of lead occurs. All fat was extracted with ether from the dry bone meal, which was then boiled repeatedly

with distilled water until the liquor gave only a very faintly positive biuret test. Shaking the prepared bone meal with lead chloride solution in a shaking machine was found to be objectionable because a certain amount of the bone meal became pulverized and had to be removed by filtration. It is preferable to avoid such manipulation because of the high adsorptive capacity of filter paper for lead salts. An arrangement was therefore contrived for so circulating the lead solution that it was continually exposed to the bone meal. For this purpose the circulating pump shown in Figure 2 was devised and built. In this pump the bulb *A* has a volume of about 150 c.c. The solid material rests on the surface of a porcelain plate sealed in at *B*. The liquid is circulated on the principle of an air lift by a very gentle stream of air blown into the apparatus at *D;* applying suction at *E* with *D* open will also cause circulation, but positive pressure at *D* gives much more satisfactory results. The usual rate of circulation with the pumps was approximately 160 c.c. per minute. With such an arrangement as this, the lead solution is constantly exposed to the surface of bone meal at a predetermined rate and can be separated readily without filtration by applying air pressure at *E* and collecting the liquid as it is forced over at *D*. The bone meal can be washed effectively by replacing lead solution with distilled water and forcing this off as before.

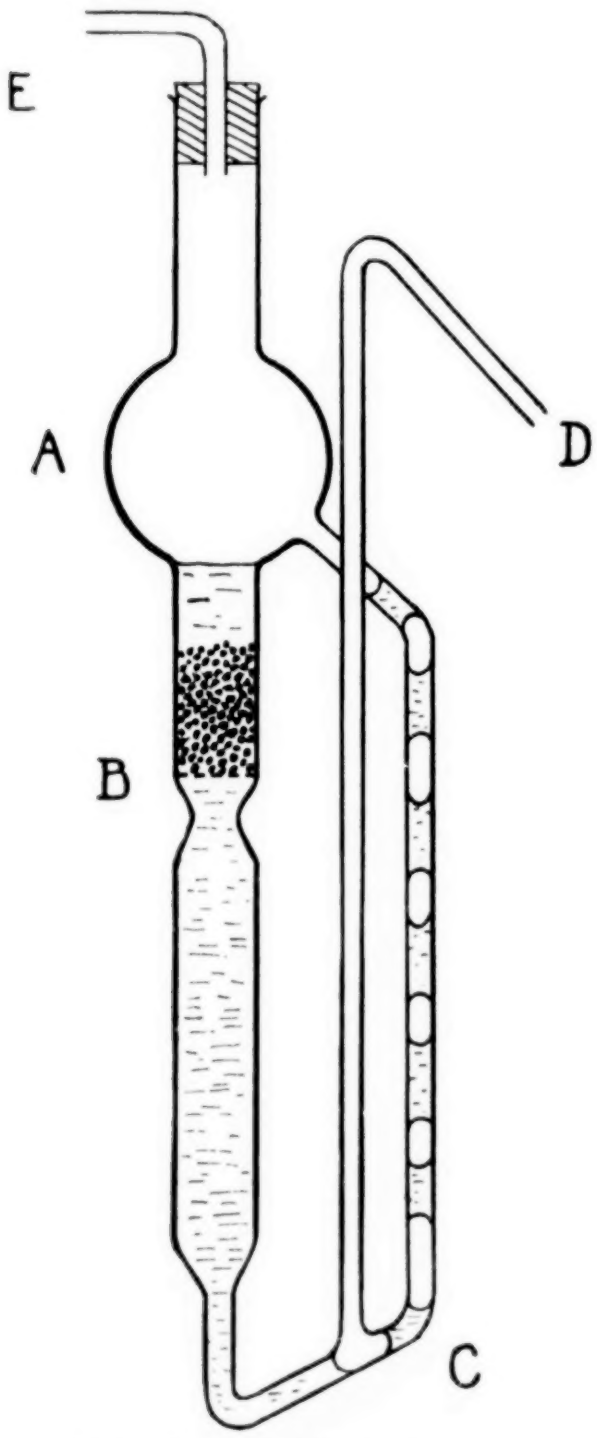


FIG. 2.—Circulating pump.

Since preliminary experiments with bone meal indicated that lead is absorbed very rapidly, it was important to discover whether the true nature of the process is replacement of calcium by lead or whether lead is directly adsorbed. Direct adsorption would be of peculiar interest, because deposition of calcium salts in the bones has been ascribed to adsorption (5, p. 750). Such aspects of absorption of lead as rate, effect of concentration, effect of changes in hydrogen ion concentration, etc., have, therefore, been carefully studied.

## RATE OF ABSORPTION OF LEAD

When an aqueous solution of lead chloride is pumped over bone particles, the amount of lead removed varies directly with the time of contact. Measurements of the amount of lead thus absorbed were made in a series of experiments in which for varying periods of time a given quantity of lead chloride was pumped over uniform amounts of crushed bone. The initial $p_H$ of the lead chloride solution was adjusted to 7.4 by means of sodium acetate solu-

tion. At the end of each period the lead solution was removed and the bone twice thoroughly washed with distilled water. The results obtained are recorded in Table 3 and shown in Figure 3 graphically. It is apparent that the

TABLE 3.—RATE OF ABSORPTION OF LEAD BY BONE

| NUMBER | INITIAL AMOUNT OF LEAD IN SOLUTION | FINAL AMOUNT OF LEAD IN SOLUTION | TIME |
|---|---|---|---|
| | *mg.* | *mg.* | *min.* |
| 1 | 25 | 15.89 | ¼ |
| 2 | 25 | 10.78 | 1 |
| 3 | 25 | 6.64 | 2 |
| 4 | 25 | 4.44 | 3 |
| 5 | 25 | 1.49 | 6 |
| 6 | 25 | 0.30 | 9 |
| 7 | 25 | 0.12 | 12 |
| 8 | 25 | 0.00 | 15 |
| 9 | 25 | 0.00 | 30 |

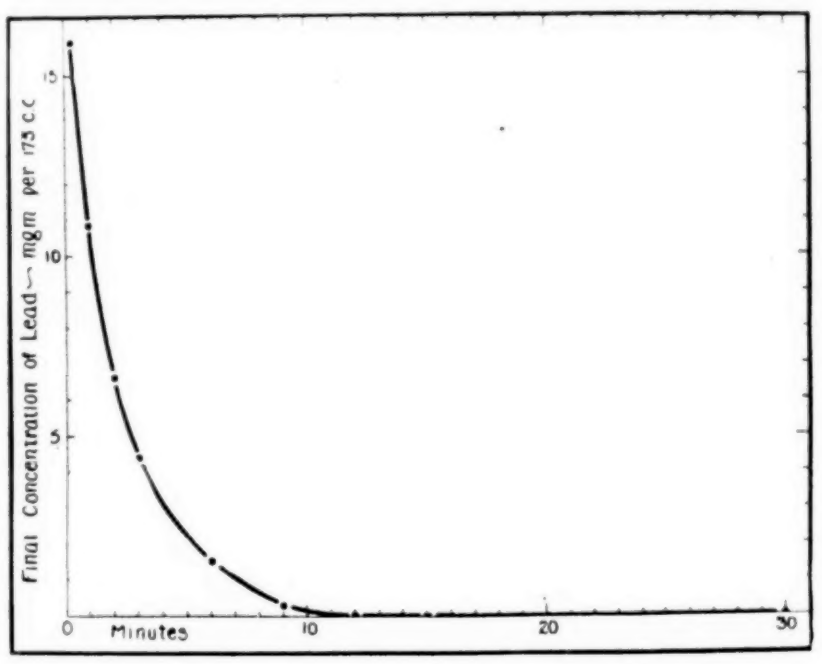


FIG. 3.—Rate of absorption of lead by bone.

majority of the lead is taken from solution in less than ten minutes. This rapid removal of lead, which is characteristic of all the experiments with crushed bone, indicates the effectiveness of the mechanism by which lead may be taken from solution.

## EFFECT OF HYDROGEN ION CONCENTRATION

The rate of absorption of lead salt by bone meal is markedly affected by the hydrogen ion concentration of the solution. Small irregularities in the rate of absorption noted during earlier experiments were attributed to slight differences in acidity arising in part from hydrolysis of the lead chloride. But when the lead chloride solutions always had exactly the same $p_H$, consistent results were obtained. Since this also has an important bearing upon absorption of lead in general, the effect of variations in hydrogen ion concentration was investigated. All other factors, such as time of contact, concentration of solution, etc., were kept constant, while only the initial hydrogen ion concentration of the lead chloride solution varied. The acidity of the solution was adjusted by the addition of sodium acetate and lactic acid or sodium hydroxide. The latter was used only for points above $p_H$ 7.8.

TABLE 4.—EFFECT OF HYDROGEN ION CONCENTRATION UPON ABSORPTION OF LEAD BY BONE

| NUMBER | INITIAL AMOUNT OF LEAD IN SOLUTION | FINAL AMOUNT OF LEAD IN SOLUTION | $p_H$ |
|---|---|---|---|
| | *mg.* | *mg.* | |
| 1 | 125 | 72.3 | 4.0 |
| 2 | 125 | 45.4 | 5.0 |
| 3 | 125 | 34.8 | 6.0 |
| 4 | 125 | 16.8 | 6.8 |
| 5 | 125 | 12.3 | 7.2 |
| 6 | 125 | 10.8 | 7.4 |
| 7 | 125 | 7.8 | 7.6 |
| 8 | 125 | 6.5 | 7.8 |
| 9 | 125 | 15.1 | 8.0 |

It is clear from the figures in Table 4 and Figure 4 that the greater the hydrogen ion concentration the less lead is absorbed—particularly at $p_H$ 4.0 where less than 43 per cent. of the total is removed from solution. As the alkalinity increases beyond $p_H$ 7.8 there is again a decrease in the amount of lead absorbed, doubtless because of the solvent action of the sodium hydroxide. It is interesting biologically that the range of maximum absorption is between $p_H$ 7.4 and $p_H$ 7.8.

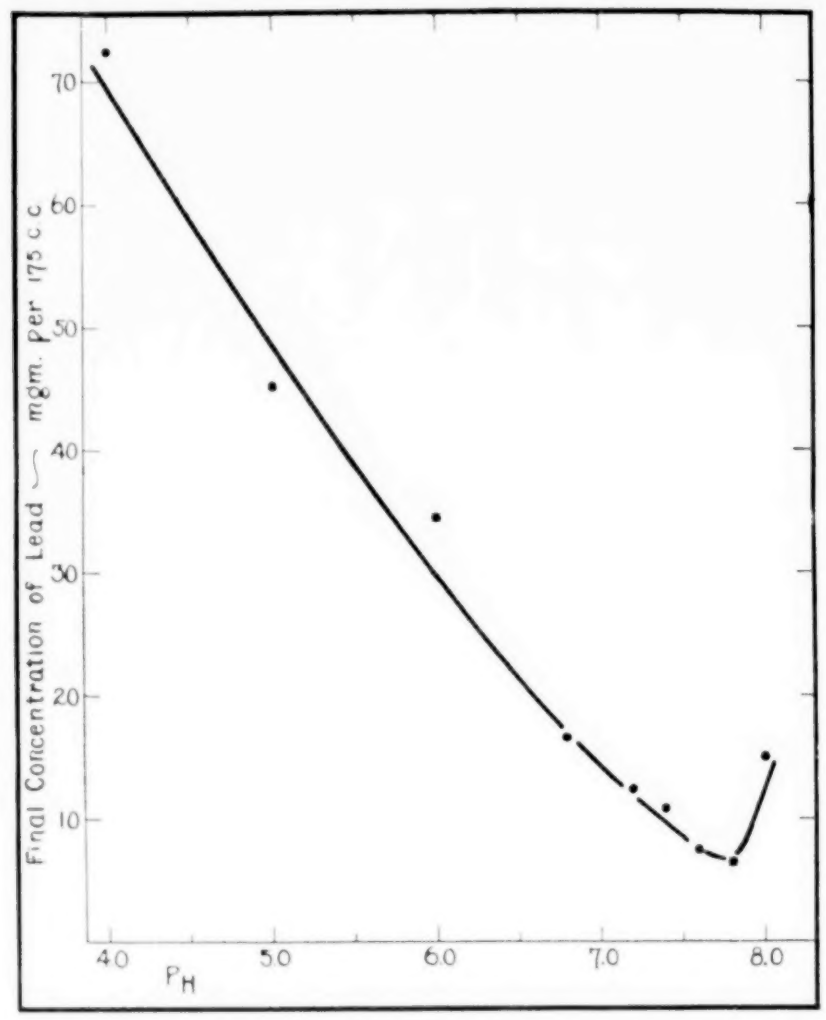


FIG. 4.—Effect of hydrogen ion concentration upon absorption of lead by bone.

## THE RELATIVE ABSORPTION OF LEAD BY VARIOUS SUBSTANCES

Three types of experiments were performed in order to determine whether lead removed from solution by bone replaces the calcium of the bone in the form of phosphate or whether it unites directly with the protein material which constitutes the organic matrix of bone. (*a*) Finely crushed bone was ignited in an electric muffle furnace to remove all organic material. The inorganic residue, which still retained the original shape of the bone particles, was then treated with lead chloride solutions of varying concentrations. (*b*) Bone particles of the same size were decalcified with dilute hydrochloric acid, thoroughly washed with water and finally with a very dilute solution of sodium carbonate and water, and then mixed with lead chloride solutions to determine their power of absorption. The $p_H$ of the solution was adjusted to 7.4. (*c*) The degree of absorption of lead from a lead chloride solution by kelp charcoal was also determined at the same concentrations. The results of these different experiments are given in Table 5 and are also graphically shown in Figure 5.

The relative difference between absorption of lead by crushed and ignited bone on the one hand and by decalcified bone and kelp charcoal on the other is clearly demonstrated. The last two cases are apparently examples of true adsorption, while the first two illustrate chemical replacement of calcium by lead. When sections of bone which has been exposed to lead are stained with ammonium sulphide and examined microscopically, lead sulphide may be seen as a dense compact layer on the surface of the bone. Sections of decalcified bone which have been similarly treated demonstrate deep penetration of the lead.

TABLE 5.—ABSORPTION OF LEAD BY CRUSHED, DECALCIFIED, AND IGNITED BONE, AND BY KELP CHARCOAL

| NUMBER | INITIAL AMOUNT OF LEAD IN SOLUTION | AMOUNT OF LEAD IN SOLUTION AFTER 30 MINUTES | | | |
|---|---|---|---|---|---|
| | | CRUSH'D BONE | DECALCIFIED BONE | IGNITED BONE | KELP CHARCOAL |
| | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| 1 | 25 | 0.00 | 3.72 | 0.00 | 4.77 |
| 2 | 50 | 0.40 | 10.09 | 0.00 | 20.45 |
| 3 | 75 | 0.89 | 27.64 | 0.00 | 10.12 |
| 4 | 100 | 7.23 | 45.48 | 0.77 | 56.06 |
| 5 | 125 | 8.05 | ..... | 2.96 | ..... |
| 6 | 150 | 17.50 | 89.99 | 11.03 | 90.71 |
| 7 | 200 | 42.00 | ..... | 19.42 | ..... |

Since normal bone removes lead rapidly and completely from solution, it was of interest to determine whether an equivalent quantity of calcium is set free during this process of absorption. Solutions of lead chloride of varying concentrations were circulated over known amounts of crushed bone for varying lengths of time and then analyzed for lead and calcium. In a series

of control experiments sodium chloride solutions of equivalent concentrations were pumped over the same amounts of crushed bone—care being taken to maintain the same hydrogen ion concentration—and the amount of calcium thus taken into solution was determined. This amount was then deducted from the amount of calcium recovered from solutions of lead chloride. The average correction was $3 \times 10^{-4}$ mols per liter. Table 6 gives the results obtained in the different experiments.

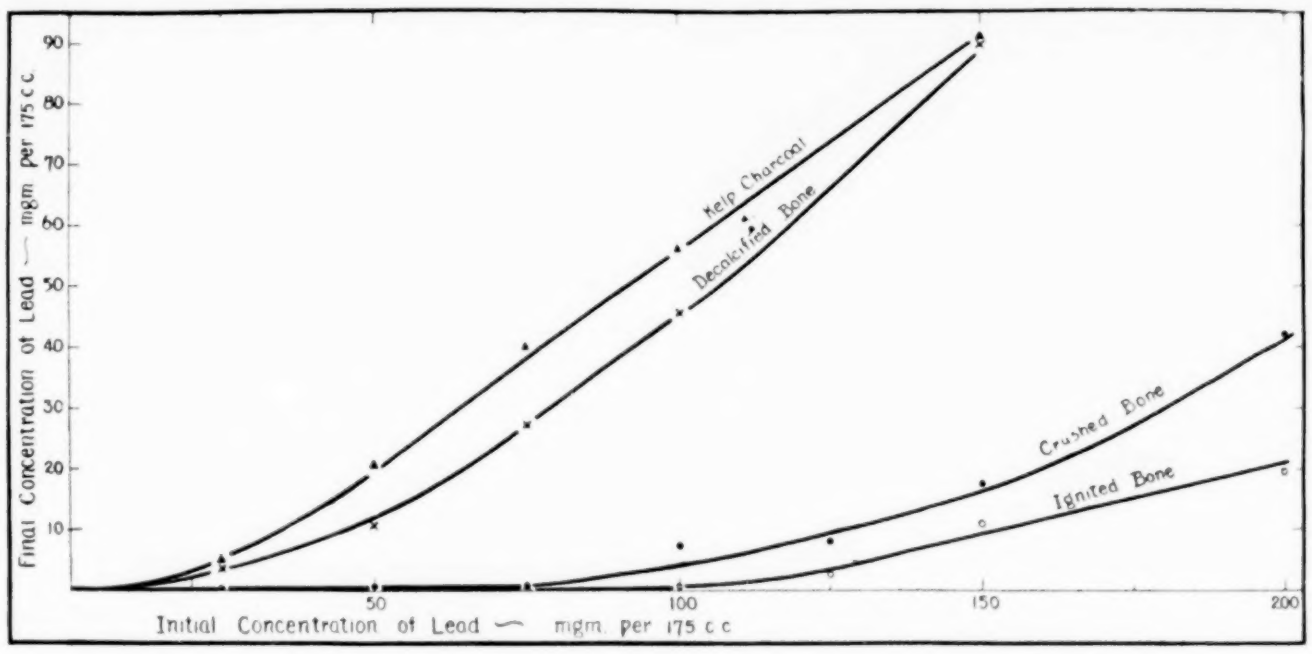


FIG. 5.—Absorption of lead by crushed, decalcified, and ignited bone, and by kelp charcoal.

TABLE 6.—REPLACEMENT OF CALCIUM IN BONE BY LEAD

| NUMBER | INITIAL MOLAR CONCENTRATION OF LEAD x 1,000 | MOLS OF LEAD ABSORBED x 1,000 [a] | MOLS OF CALCIUM LIBERATED x 1,000 [b] | [a]/[b] |
|---|---|---|---|---|
| 1 | 1.38 | 1.36 | 1.09 | 1.25 |
| 2 | 2.07 | 2.05 | 1.82 | 1.12 |
| 3 | 2.78 | 2.66 | 2.08 | 1.28 |
| 4 | 3.45 | 3.22 | 2.28 | 1.41 |
| 5 | 4.14 | 3.80 | 2.41 | 1.57 |
| 6 | 5.52 | 4.36 | 2.74 | 1.59 |

$P_H = 7.4$. Volume = 175 c.c.

Figure 6 shows the existence of a definite relationship between the calcium and lead exchanges although the two curves do not exactly coincide. Chemical replacement of the calcium by lead is demonstrated by the constantly the solution remains practically uniform throughout the experiments.

Pfaundler (8) observed that cartilage removes more calcium than chlorine from a solution of calcium chloride and suggested a specific ionic affinity as explanation. That crushed bone removes lead from a solution of lead chloride is true, but this reaction is one of chemical replacement. In order to determine whether chloride is also removed, analyses were made for chlorine during absorption. A solution of sodium chloride practically equivalent in concentration to the solution of lead chloride was analyzed and found to contain 8.57 mg.

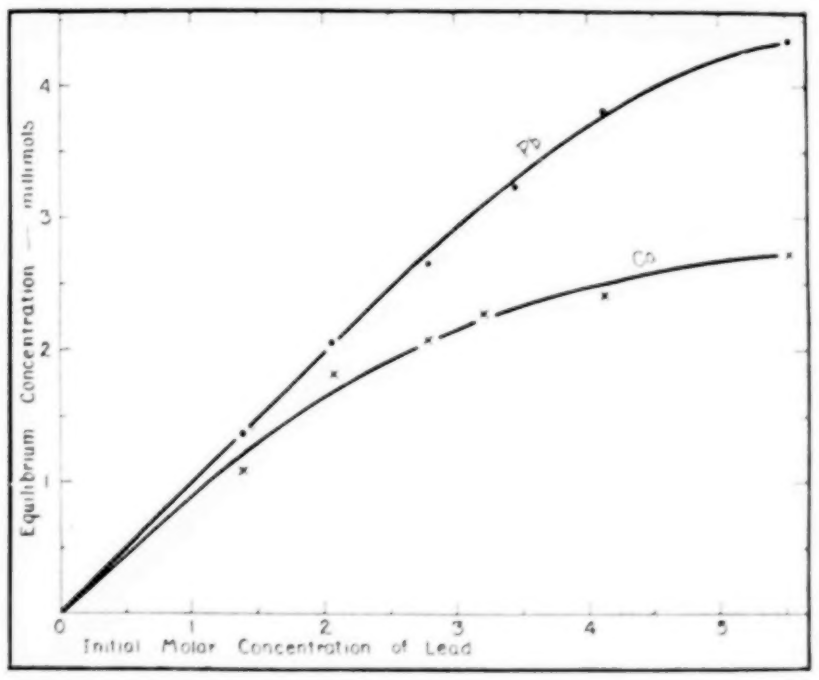


FIG. 6.—Chemical replacement of calcium in bone by lead.

of chlorine per 100 c.c. After being circulated over purified crushed bone the solution was again analyzed, and the chlorine content was found to be 8.60 mg. per 100 c.c. A solution of lead chloride (8.04 mg. of chlorine per 100 c.c.) was then circulated over another sample of the same bone until the lead was completely absorbed. Analysis of this solution and the washings showed that 8.10 mg. of chlorine per 100 c.c. remained in solution. It is, therefore, clear that there is no change in the chloride content and that the calcium of bone is merely substituted for lead in the solution.

### The Absorption of Lead by Cartilage

Numerous attempts have been made to simulate the formation of bone by the interaction of cartilage and calcium salts. Freudenberg and György (9), who carried out an extensive study of this problem on the basis of earlier work by Pfaundler, found that cartilage immersed in calcium chloride solution absorbed the calcium in amounts which were determined by the hydrogen ion concentration. The more acid the solution, the less calcium was removed. They also found that cartilage took magnesium, barium, strontium, etc., from solution. But it appears that even when the time of contact was very prolonged, absorption was not complete.

Similar experiments in this laboratory have shown that lead is removed from solution by fresh cartilage but not so efficiently as by crushed bone. The quantity absorbed is directly proportional to the time of contact and varies with the hydrogen ion concentration of the solution. For example, 15 gm. of fresh finely chopped cartilage from the nasal septum of an ox, mixed with Ringer solution containing 25 mg. of lead, removed 79 per cent. of the lead from solution in two hours. This cartilage impregnated with lead gives up its lead much more readily in acid solution than does crushed bone after the same treatment. If the lead combines chemically with the protein material of the cartilage this should be expected, because lead albuminate, which is very soluble in acid medium, dissolves at hydrogen ion concentrations slightly above $p_{H}$ 5.8.

The experiments with crushed bone do not necessarily demonstrate that lead is taken from the circulating blood stream *in vivo* by direct chemical replacement; but they do both explain the removal of lead by bones, dried or freshly dissected from the animal, and emphasize the sensitiveness of absorbed lead to changes in hydrogen ion concentration.

### The Transportation and Deposition of Lead in the Body

Soluble lead salts readily dissolve in an excess of blood plasma; and far larger quantities may be held in solution than would dissolve in an equal quantity of water containing the same concentration of inorganic salts. It is commonly supposed that lead in the blood stream is in the form of albuminate, but it is more probably present as highly dispersed phosphate, one of the most insoluble lead salts. The behavior of this is like that of the tricalcium phosphate which is found in mammalian blood in quantities two to four times larger than would dissolve in pure water. A similar dispersion of a lead salt can be shown in the formation of lead sulphide in serum. Instead of the precipitation of the sulphide as a black solid, there is merely a change of color to clear red. This indicates

that the sulphide forms, but in a highly dispersed or colloidal state. Lead salts may be thus dispersed or peptized by various substances. For instance, a suspension of lead phosphate in a solution of cane sugar may be prepared without any trace of opalescence, while the same quantity of phosphate in pure water has a milky appearance. If hydrogen sulphide is added to the cane sugar suspension, the color changes to red just as in the case of phosphate and blood serum. Distribution experiments have shown that the greater part of the lead transported by blood is in the plasma and that the cells apparently carry but little. The blood of a rabbit with lead poisoning was found to have 79 per cent. of the total lead in the plasma. In a fatal case of lead encephalopathy the concentration of lead in the blood was recently found to be 2.80 mg. per liter—an amount which corresponds to 3.64 mg. of tri-lead phosphate. As the solubility of this salt is only 0.13 mg. in a liter of pure water (10) the quantity of lead salt in the blood was about thirty times as great as the amount which would dissolve in a corresponding volume of water. Since lead could exist for a long time in the presence of inorganic salts of the blood only as the correspondingly most insoluble lead salt, *e.g.*, lead phosphate, and since lead phosphate is so insoluble, it seems reasonable to believe that lead is transported by the blood as highly dispersed phosphate.

Any satisfactory mechanism for the deposition of either lead or calcium salts in the organism is difficult to conceive. Probably the most stimulating hypothesis so far advanced has been that of adsorption of *dissolved* calcium salts (5). If adsorption is to explain the deposition of lead salts by the organic matrix of intracellular substance, however, the process must be adsorption of *colloidal* lead phosphate. Illustrations of adsorption of a colloid from a liquid are numerous; for instance, colloidal gold is adsorbed by barium sulphate or aluminium hydroxide. When borne by the circulating blood to the hyaline matrix where slight changes in alkalinity probably initiate the adsorption of calcium salts, colloidal lead phosphate would probably be deposited. The precipitation of such a colloid occurs much more rapidly than the dissolving of lead phosphate or the dispersion of the lead salt in a medium containing an excess of precipitant. The deposition of lead in bone tissue in the case of a rabbit injected intravenously with a lead salt solution amounted to 24.3 mg. in seven hours, or 30 per cent. of the total amount injected. This rapid rate of storage is interesting in view of the hypothesis that lead is deposited in the body by a process of adsorption of colloidal lead salts.

## Summary

Absorption experiments have been made with crushed bone, cartilage, decalcified and ignited bone, and charcoal under varying conditions of concentration, time, and acidity. These show that lead is quantitatively removed from solution within a very short time by means of crushed bone; and that deposited lead is sensitive to changes in acidity. The maximum deposition occurs at $p_H$ 7.4 to 7.8. *In vitro* experiments show that under certain conditions lead chemically replaces the calcium in bone.

The solubility of di-lead phosphate in water at 25° C. was determined and found to be 0.0129 gm. per liter. The solubilities of di-lead phosphate and of tri-lead phosphate in various concentra-

tions of lactic acid were measured. The tri-lead phosphate proved to be much more soluble in lactic acid than the di-lead salt.

Although there are points of similarity, experiments have indicated that the deposition of lead salt in living bone tissue is probably not entirely analogous to the absorption of lead by bone *in vitro*. It has been shown how the hydrogen ion concentration may be an important factor in governing the amount of lead in the circulation.

## BIBLIOGRAPHY


1. Minot, A. S.: Lead Studies V. The Distribution of Lead in the Organism. THIS JOUR., 1924-1925, *6*, 125, 137, 149.
2. König, J.: Substitution des Kalkes in den Knochen. Ztschr. f. Biol., 1874, *10*, 69.
3. Lehnerdt, F.: Zur Frage der Substitution des Calciums im Knochensystem durch Strontium. Beitr. z. path. Anat. u. z. allg. Path., 1909, *46*, 468; 1909-1910, *47*, 215.
4. Stoeltzner, H.: Ueber den Einfluss von Strontiumverfütterung auf die chemische Zusammensetzung des wachsenden Knochens. Biochem. Ztschr., 1908, *12*, 119.
5. Wells, H. G.: Calcification and Ossification. Arch. Int. Med., 1911, *7*, 725.
6. Fairhall, L. T.: Lead Studies VII. The Phosphates of Lead. Equilibrium in the System Lead Oxide-Phosphoric Anhydride-Water at 25°. Jour. Am. Chem. Soc., 1924, *46*, 1593.
7. Alders, H., and Stähler, A.: Zur Kenntnis der Bleiphosphate. Ber. d. deutsch. chem. Gesellsch., 1909, *42*, 2261.
8. Pfaundler, M.: Ueber die Elemente der Gewebsverkalkung und ihre Beziehung zur Rachitisfrage. Jahrb. f. Kinderheilk., 1904, *60*, Ergnzngshft., 123.
9. Freudenberg, E., and György, P.: Ueber Kalkbindung durch tierische Gewebe. Biochem. Ztschr., 1920, *110*, 299; 1921, *115*, 96; *118*, 50; *121*, 131, 142; *124*, 299; 1922, *129*, 134, 138.
10. Landolt - Börnstein: Physikalische - Chemische Tabellen. Berlin, 1923, p. 679.